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Breeding for Nitrogen Use Efficiency in Soft Red Winter Wheat

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BREEDING FOR NITROGEN USE EFFICIENCY IN SOFT RED WINTER WHEAT

THESIS

A thesis submitted in partial fulfillment of the
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ABSTRACT OF THESIS

BREEDING FOR NITROGEN USE EFFICIENCY IN SOFT RED WINTER WHEAT

Nitrogen use efficient (NUE) wheat varieties have potential to reduce input costs for growers, limit N runoff into water ways, and increase wheat adaptability to warmer environments. Previous studies have done little to explain the genetic basis for NUE and components, nitrogen uptake efficiency (NUpE) and nitrogen utilization efficiency (NUtE). Four studies were conducted to 1) determine genotypic stability of NUE under high and low N regimes and under warming 2) determine effect of warming on NUE 3) indentify QTL associated with NUE components 4) assess the utility of canopy spectral reflectance (CSR) as a high-throughput phenotyping device for NUE. Genotypic response to N stress or warming varied. Uptake efficiency was found to be more important than utilization efficiency to genotypic performance under high and low N environments and under warming. Selection under low N for NUpE and under high N for NUtE most efficiently identified NUE varieties. Uptake and utilization were lower under warming due to quickened development. No strong correlations between the CSR indices and NUE existed. No QTL were found to be significantly associated with NUE components. Further research into the mechanisms controlling NUE and to reveal plant response to N stress and under warming is necessary.

KEYWORDS: Winter wheat, nitrogen use efficiency, nitrogen uptake efficiency, nitrogen utilization efficiency, warming

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Summary

Nitrogen-use efficient crop varieties may ensure sustainability in agricultural systems and meet future consumer demands, especially when faced with changing environments caused by climate change. Nitrogen-use efficient varieties may decrease environmental and economic costs associated with excess fertilizer N. Wheat varieties that take up and utilize nitrogen more efficiently may be more adaptable to warmer environments since grain filling can continue under stress conditions when photosynthesis is compromised. This thesis reports on four studies: Ch. 3: Using High-Throughput Phenotyping through Canopy Spectral Reflectance to Estimate Nitrogen Use Efficiency in the Soft Red Winter Wheat Elite Mapping Panel; Ch. 4 Effects of Warming on Nitrogen Use Efficiency at Different N Rates in Soft Red Winter Wheat; Ch. 5 Selecting for Nitrogen-Use Efficient Soft Red Winter Wheat Lines Under High and Low Nitrogen Environments; Ch. 6 Breeding for Nitrogen Use Efficiency to Combat Heat Stress Caused by Climate Change.

Results from these studies demonstrate genotypic variation in the two components of N use efficiency, N uptake efficiency and N utilization efficiency. Response to N stress or warming varied among the genotypes. In the active warming study (Ch. 6), principal component analysis showed that uptake and utilization efficiency accounted for most of the variation observed within the data. Across the four studies uptake efficiency was found to be more important than utilization efficiency to genotypic performance in terms of yield, N use efficiency, and N grain content under high and low N environments and under warming. Genetic variation for the low N environments from the hill plot study 2013 (Ch. 4) and N plot study 2014 (Ch. 5) was found to be greater than under the high N environments for traits such as yield, N use efficiency, and N grain content. Thus, selection for high yielding, high quality lines may be more accurate under N limiting environments because the true genotypic performance is masked under N sufficient environments. Under low N, genetic variation was higher for uptake efficiency, while variation in utilization efficiency was higher with high N. Therefore, selection in low N environments for the former and under high N environments for the latter should be

implemented to identify varieties that have high uptake efficiency and that have high utilization efficiency that results in increased yield and NUE. Post-anthesis N uptake (PANU) had significant positive correlations to yield, grain N content, and uptake efficiency across N environments and locations in chapters 4 and 5 in high N environments.

Nitrogen use efficiency was also found to be affected by increased temperatures (Ch. 4 and 6); both uptake and utilization were lower under increased temperatures. In wthe warmed environment, development was quickened. Accelerated development may have reduced the length of time allowed for N remobilization to the grain, causing more N to be left in the biomass, thus causing a reduction in yield and grain N content. Increases in yield ranking and stability under warming were largely related to increases in uptake efficiency. However, there were a few genotypes that increased performance due to increases in utilization efficiency or both utilization and uptake efficiency under warming. Results from the warming study (Ch. 6) suggest that uptake efficiency may be more important in producing yield and grain N content under warming in our region. Selecting for uptake efficiency and related traits may be a good strategy to increase overall NUE, yield, and grain N content. Lines with higher uptake efficiency may be able to maintain yield and quality under warmer environments and can be incorporated into breeding programs developing genotypes adapted to future climates.

While non-destructive techniques like canopy spectral reflectance (CSR) offer promising ways to phenotype traits such as N use efficiency (Ch. 3 and 5), in my studies, there were not strong correlations between the CSR indices, NDVI and RIRR, and yield and N traits. Nitrogen use efficiency is a complex trait that is influenced by the environment and was shown to have low heritability; thus, being able to find markers linked to it would be very beneficial to breeding programs. However, the association analysis performed in TASSEL to find markers related to the N traits was not fruitful. Some markers showed association to N traits, but these associations were not significant according to the Bonferroni test correction. The lack of significant markers was likely due to the small number of genotypes used to perform the analysis. To gain further insight into the mechanisms controlling NUE under adverse environmental conditions and to reveal plant response to N stress and warming, other tools such as

ecophysiological models and crop models could be incorporated. Using a larger population to identify QTL for N use efficiency and its components using molecular methods and association mapping could improve development of N use efficiency. Further research is needed integrating genetics and physiology to discover the location of QTL linked to N uptake and N utilization efficiencies to better understand the way in which alleles associated with QTL may influence differences in N use efficiency in winter wheat.

Chapter 1

Introduction

Wheat (*Triticuum aestivum* L.) is an important component of the national and global food supply. Soft red winter wheat is the 4th major crop grown in Kentucky. Earth's changing climate has the potential to adversely affect this important crop due to increasing temperature. Rising levels of greenhouse gases are causing Earth's temperature to increase. CO_2 concentrations in Earth's atmosphere have risen since 1800, increasing from 280 to 390 mmol CO_2 per mol of atmosphere and climate models indicate these values will only rise in the future (Bloom *et al.*, 2010). Historic data indicate that from the 1970's to present day each decade has been warmer than the past 100 years (IPCC, 2007). Projections show that temperatures will rise in the future, 1.4-4.8^{o}C by 2050 (IPCC, 2001 and 2007). In wheat, temperatures greater than 30^{o}C can cause the plant to experience heat stress and cause reductions in grain number or grain filling period, resulting in yield losses. The yield component that is affected depends on the developmental stage at which the increased temperature event occurs. Elevated temperatures before anthesis result in decreased grain number. If the temperature event occurs after anthesis, grain filling duration is shortened (Ferris *et al.*, 1998).

Agricultural crops, including wheat, are inefficient at uptaking and utilizing fertilizer nitrogen. As a result, the nitrogen is lost through processes such as leaching or dentrification which can reduce the health of the surrounding environment. Loss of nitrogen through leaching can reduce water quality and health of freshwater and marine ecosystems through eutrophication (Sieling and Kage, 2008). Nitrogen fertilizer can also be lost to the atmosphere as nitrous oxide, a potent green house gas. In the U.S., agriculture is estimated to contribute 8.6% of green house gas emissions, 80% being from nitrous oxide. Worldwide, agriculture represents 13.5% of anthropogenic green house emissions (Karl *et al.*, 2009).

In the U.S., wheat yield gains have slowed since the 1990's (Muurinen *et al.*, 2007). As human population growth continues, worldwide demand for wheat will continue to increase (Ludwig and Asseng, 2006; Parry and Hawkesford, 2012). Thus,

providing food for future populations while assuring sustainability of agricultural systems is becoming an increasing dilemma. To achieve this goal in agricultural production, other obstacles such as climate change must be oversome. Nitrogen-use efficient crop varieties may be a good option to ensure sustainability in agricultural systems and meet future consumer demands, especially when faced with changing environments caused by climate change. Nitrogen-use efficient crop varieties have the potential to decrease environmental and economic costs associated with adding additional nitrogen to the soil. In wheat and in other crops, lines that utilize nitrogen more efficiently may have the ability to withstand the adverse affects from increased temperature caused by climate change since theses plants are able to take up more soil nitrogen and continue grain filling under stress conditions when photosynthesis is compromised. Therefore, a series of studies was conducted to determine: 1) nitrogen use efficiency of soft red winter wheat varieties grown in Kentucky under adverse field conditions (N limiting/temperature stress), 2) how this trait was affected under these conditions, and 3) which traits may be associated with nitrogen-use efficiency.

Chapter 2

Literature Review

Importance of Nitrogen Use Efficiency (NUE)

Nitrogen (N) is a critical nutrient for canopy growth, and canopy photosynthesis drives grain yield and grain quality (Hawkesford, 2012). Excess nitrogen has been shown to have adverse environmental impacts, such as eutrophication of freshwater and marine ecosystems that occurs when high quantities of N fertilizer are added to soil and then washed into the stream through runoff (Sieling et al, 2009). However, reduction in fertilizer use could also decrease crop yield and quality if the plant experiences N deficiency (Cassman *et al.*, 2003). Therefore, great interest has been focused on crop varieties with high nitrogen use-efficiency because these plants would be expected to minimize environmental and production costs associated with addition of excess N to agricultural systems. Increasing NUE can also reduce the amount of greenhouse gases emitted by the crop, associated with the production of grain yield (Gaju *et al.*, 2011). Global use of nitrogen fertilizer has steadily increased, along with agricultural emissions to the environment, predominantly as nitrous oxide (Raun et al., 2001; Tilman *et al.*, 2001; Muurinen *et al.*, 2007; Hatfield *et al.*, 2012). However, even though worldwide fertilizer use has increased, NUE and yield have not risen in cereal crops such as wheat (Hatfield and Prueger, 2004; Muurinen *et al.*, 2007). In wheat production, 70% of N fertilizer accounts for increased greenhouse gases as nitrous oxide (Mortimer *et al.*, 2004). Some climate models have shown that NUE crops could decrease nitrous oxide gas release, thus reducing green house gas emissions from addition of N fertilizer. As a result, selecting and developing NUE crops has gained momentum among breeders (IPCC, 2010). However, breeding NUE crops can be complicated especially since NUE can be described in a variety of ways.

Some researchers have measured or calculated traits, such as nitrate uptake, grain protein content, harvest index (HI), or nitrogen harvest index (NHI) to characterize NUE and select for N efficient genotypes (Berry *et al.*, 2010; Cregan and Van Berkum, 1984; Schulte auf'm Erley *et al.*, 2011; Nyikako *et al.*, 2014). Others have described varieties

that are N-use efficient as being able to produce higher than average yields in low N environments (Nyikako *et al.*, 2014). Nitrogen use efficient varieties have also been characterized as genotypes that are able to generate higher yields when additional N was provided. Several authors have suggested that nutrient stress response should be accounted for when estimating NUE (Lobell and Ortiz-Monasterio, 2007). Additionally, NUE has been defined as the amount of biomass produced divided by the amount of soil N supply (Soil N + fertilizer N applied) (Good *et al.*, 2004; Hawkesford, 2012; Nyikako *et al.*, 2014). The most practical definition of NUE for breeding describes NUE as the grain yield divided by the soil N supply (soil N and fertilizer N) (Moll *et al.* 1982). This definition incorporates the two components of NUE, N uptake efficiency (NUpE) (ability of the plant to take up soil N) and N utilization efficiency (NUtE) (ability of the plant to generate yield from N accumulated in vegetative tissue) (Moll *et al.*, 1982; Nyikako *et al.*, 2014). Nitrogen uptake efficiency can be calculated by dividing total plant N by the soil N supply and NUtE can be measured by dividing yield by the total plant N. Total plant N is the amount of N in the aboveground material at maturity (grain N content (yield * % N grain) + N content in straw (biomass * % N straw) (Moll *et al.*, 1982).

Genetic gains in NUE are dependent upon the two components of NUE, NUpE and NUtE. For instance, Ortiz-Monstarerio (1997) found that increases in NUE were attributed to NUpE under low N conditions in Mexico. Researchers in Finland observed similar trends under low N environments (Muurinen *et al.* 2006). However, studies conducted in other parts of Europe found that NUtE had greater influence over NUE in low N environments (Foulkes *et al.* 1998; Brancourt-Hulmel *et al.*, 2003). In high N environments, in areas such as UK, Mexico, and Finland, researchers found that both NUtE and NUpE had an equal affect on NUE increases in wheat (Ortiz-Monstarerio *et al.* 1997, Foulkes *et al.* 1998, Muurinen *et al.* 2006). Other studies have shown that NUpE caused greater genetic variation of NUE under low N than high N conditions (LeGouis *et al.*, 2000). However, a study done in California using 12 spring wheat lines observed that NUpE was the strongest factor in determining NUE at both low and high N supply (Dhugga and Waines, 1989). An experiment using 5 different N rates found that wheat genotypic differences in NUE were associated with NUpE at the highest N rates and NUtE explained more of the differences in grain yield than NUpE across all 5 N rates

(Barraclough *et al.*, 2010). Overall, the main effects observed from these studies were the interaction of N supply and genotype on yield and NUE. Also, under low N environments yield and NUE could be influenced by NUpE or NUtE depending on the site, unlike high N environments where yield and NUE were almost always more closely related to NUtE (Gaju *et al.*, 2011). These studies used 12-24 genotypes; therefore, using a wider range of wheat genotypes may be more useful for selection of high yield and quality wheat lines under different N environments at multiple locations.

Traits Related to NUE Under Adverse Field Conditions

Around the world, most wheat varieties are tested under high N environments. This means plant breeders select varieties that perform well under these conditions. However, genotypes selected for high yield in these environments may not produce high yields under low N environments, since studies have shown an interaction between genotype and N supply (Ortiz-Monstarerio *et al.* 1997, Foulkes *et al.* 1998, Muurinen *et al.* 2006). Therefore, breeding and selection in low N environments may be needed to appropriately identify varieties with superior yield and NUE (Brancourt-Hulmel *et al.*, 2005). A knowledge of which traits are important for NUE under these N conditions and warming is necessary to identify efficient cultivars. For instance, some studies have shown a relation between final grain N and NUpE. Root morphology, nitrate reductase activity, root hair development, and presence of arbuscular mycorrhizae likely affect NUpE (Baresel *et al.*, 2008). Therefore, root growth may influence overall NUE. Development of new roots is necessary for the plant to make use of resources in unexplored soil and the path of root formation may vary among genotypes, and thus root traits are likely important in determining crop NUpE and overall NUE (Baresel *et al.*, 2008).

During development, N source-sink-relationships change, the critical point being anthesis. After anthesis, N taken up by the roots and stored in the vegetative tissue is remobilized to the grain, making the grain the primary sink (Simpson *et al.* 1983; Baresel *et al.*, 2008). Therefore, N partitioning can be defined as uptake before anthesis, post-anthesis N uptake, and remobilization processes (Wetselaar and Farquhar 1980). Genetic variability among root traits and source-sink-relationships has been documented

(Barbottin *et al.* 2005; Kichey *et al.* 2007; Baresel *et al.*, 2008). These traits may be used to improve adaptation to adverse environmental conditions, such as warming or N limiting environments.

Wheat N partitioning during vegetative growth, anthesis, grain filling, and maturity can be valuable. Measuring total N uptake during vegetative growth can be indicative of early plant development, tillering, and root formation (Sylvester-Bradley *et al.*, 2009; Swain *et al.*, 2014). At anthesis, measuring total N uptake can give insight into growth of yield generating leaves, floret fertility, and amount of stem reserves. Nitrogen accumulation at this developmental stage is reliant on the extent of the rooting system and N availability. At the end of anthesis, biomass partitioning can indicate preferred strategies in N storage and translocation (Cox *et al.* 1986; Swain *et al.*, 2014). Measuring total N uptake at maturity provides information on the translocation efficiency of N from the biomass to the grain. Wheat genotypes that have superior N uptake, N storage, and N translocation capabilities will allow for further gains in NUE. Other research has suggested that high NUE genotypes are those that possess the "stay green" trait which hinders senescence and allows for a longer grain filling period through the continuation of N uptake and translocation (Bogard *et al.* 2011; Swain *et al.*, 2014).

Canopy Spectral Reflectance to Identify Nitrogen Use Efficient Wheat Genotypes

More rapid and efficient selection for high yielding and stress tolerant plants may be possible through high throughput phenotyping. There have been great advances in genotyping technologies over the years (Winterhaltera *et al.*, 2010). However, deficiencies in phenotyping abilities reduce the capacity to accurately assess the genetics governing quantitative traits (Winterhaltera *et al.*, 2010; Montes *et al.*, 2011). Using high throughput phenotyping techniques can possibly connect the genotype and phenotype. Canopy spectral reflectance (CSR) devices can be utilized to implement high throughput phenotyping for complex traits, such as nitrogen concentration, associated with NUE (Li *et al*., 2014). Canopy spectral reflectance devices measure the amount of light reflected/absorbed by the plant's canopy surface. Genotypic variation and environmental stress can affect the amount of light reflected. Thus, the use of CSR in selection for NUE may be a rapid and inexpensive option (Raun *et al.*, 2001).

Nitrogen use efficiency can be estimated through traditional soil sampling and plant tissue testing. However, traditional monitoring and evaluation of crop nitrogen can be very destructive to plant samples, involve a rigorous work load, and compromise the accuracy of data results. Therefore, CSR techniques can be used as an alternative for nondestructive and real-time monitoring of crop N status. Studies have shown that a variety of CSR indices, such as the normalized difference vegetative index (NDVI) or red infrared ratio (RIRR), have high correlations with wheat grain yield, biomass and N concentration (Ma *et al.*, 1996; Raun *et al.*, 2001; Crain *et al.*, 2012). These indices are already being utilized to manage N in the field. For instance, in China, researchers are using CSR indices to estimate N uptake of winter wheat to better manage N fertilizer application (Li *et al.*, 2012). The CSR estimates of biomass and N content can be used to estimate NUpE and NUtE. The relationship of CSR with biomass is of great interest, since biomass is an essential element related to NUE and yield (Crain *et al.*, 2012). Measuring N content through CSR techniques could also give insight into the plant's physiology and may indicate other traits to investigate that may benefit NUE, such as root structure and N partitioning. For instance, it is reasonable to infer that wheat with good NUE may have a more vast root structure that allows the plants to extract more N from the soil to be utilized in the plant (Li *et al.*, 2012; Li *et al.*, 2014).

Effects of Warming from Climate Change on Wheat Production

Winter wheat is an important crop at both the regional and global scale. Increasing temperatures from climate change may have an adverse affect on winter wheat production. Climate change is caused by increases in CO_2 concentration which can cause increase in plant production by increasing photosynthesis and water use efficiency. However, increased CO_2 levels can negatively affect plant production. Elevated CO_2 levels result in decreased grain quality by reducing plant nutrient concentrations (Rogers *et al.*, 1996; Kimball *et al.*, 2001; Ludwig and Asseng, 2006). Further release of CO_2 and other greenhouse gases into earth's atmosphere can increase temperatures, causing climates to change in the future [Intergovernmental Panel on Climate Change (IPCC), 2007]. Elevated temperatures can cause plants to experience heat stress, decreasing plant productivity (Van Herwaarden *et al.*, 1998; Ludwig and Asseng, 2006). However, the

onset of heat stress is dependent on how quickly and to what degree temperature increases occur, along with the length of time the plant is exposed to elevated temperatures (Farooq *et al.*, 2011).

Future climate models project that the earth's temperature will increase between 1.4 and 5.8°C by 2050 [Intergovernmental Panel on Climate Change (IPCC), 2001 and 2007](Keating *et al.*, 2010). Future environments will also be subjected to increased temperature variability and greater number of hot days (Farooq *et al.*, 2011). A study using a crop model linked to field data showed that for every 1°C increase in global mean temperature wheat production would decrease 6 %, resulting in a 42 Mt loss of wheat with each degree temperature increase (Nelson *et al.*, 2009; Nelson *et al.*, 2014; Van Ittersum *et al.*, 2003; Asseng *et al.*, 2014). Overall, grain yields are predicted to decline in most regions worldwide and temperature impacts may be greater and begin sooner than thought before (Asseng *et al.*, 2014; Challinor *et al.*, 2014). In order to create new genotypes adapted to future climates, greater understanding of how crops act in response to elevated temperatures and how heat stress tolerance can be enhanced is a necessity (Farooq *et al.*, 2011).

Effect of Warming on Wheat Physiology

The physiological effects that occur to the wheat plant under heat stress conditions has been well documented. Plants can respond to temperature changes through differences in metabolic activity, membrane moisture content, configuration of proteins, and cytoskeleton assembly (Ruelland and Zachowski, 2010; Farooq *et al.*, 2011). These internal responses to temperature changes can trigger adaptive processes such as production of heat shock proteins. However, heat stress or temperatures exceeding optimum growth can result in injury or permanent damage (Wahid *et al.*, 2007).

In winter wheat, temperatures greater than 14°C have been shown to cause photosynthesis rates to decrease. Prasad *et al.* (2008) found that wheat grain yield decreased as temperatures rose from 14°C to 23°C. During anthesis, heat stress can increase flower abortion (Wardlaw and Wrigley, 1994). During the reproductive stage, heat stress can lead to pollen sterility, moisture reduction in plant tissues, decreased CO_2

assimilation and increased photorespiration. High temperatures can accelerate growth (Fischer, 1980; Kase and Catsky, 1984) but in doing so reduce the phenological time from one growth stage to another (Wardlaw and Moncur, 1995; Zahedi and Jenner, 2003; Farooq *et al.*, 2011). Consequently, higher temperatures between anthesis and grain maturity cause grain yield to decrease because there is less time to attain resources. Both components of yield, grain number and grain weight, are susceptible to increased temperature (Ferris *et al.*, 1998). The developmental stage at which elevated temperatures occur will determine which component of grain yield will be affected. For example, during anthesis, temperatures above 20°C may considerably decrease grain number per spike (Saini and Aspinall, 1982). Heat stress accelerates development of the spike, reducing spikelet number, resulting in fewer grains per spike (Saini and Aspinall, 1982; Porter and Gawith, 1999; Farooq *et al.*, 2011). The most sensitive stage during reproductive growth is between the double ridge (appearance of double ridges on apex of shoot) and flag leaf stage. During this stage, florets are produced in the spikelets that form in the spike. Elevated temperatures shorten this period of time, causing spikelet number per spike and grain number per spikelet to decline (McMaster, 1997). Grain number can also be reduced during floral initiation. For instance, Fischer found that grain number per spike decreased by 4% for every 1°C increase in the 30 days preceding anthesis (1985). Because insufficient assimilates can cause the floret number to decline, assimilate availability can influence floret development and thus, grain number (Abbate *et al.*, 1995; Demotes-Mainard and Jeuffroy, 2004).

Grain filling occurs between anthesis and maturity. Thus, higher temperatures shorten the length of this period, causing grain size to decrease along with yield (Warrington *et al.*, 1977; Shpiler and Blum, 1986). During grain filling, grain size can decline by about 1.5 mg/day for every 1°C greater than 15–20°C (Streck, 2005). Heat stress tolerance in terms of effects on wheat grain number and size varies among genotypes (Farooq *et al.*, 2011). For example, one experiment studying the influence of grain characteristics in spring wheat genotypes found that 14 varieties exhibited smaller grain size despite duration and timing of the elevated temperature event (Castro *et al.*, 2007). Higher temperatures can also cause changes in the aleurone layer and endosperm cells, resulting in grain size reduction. When heat stress is not present, the aleurone layer

of a wheat grain has large cells bordering the starchy endosperm. When experiencing heat stress, the endosperm cellular structure changes and becomes denser because starch granules accumulate and become embedded in the protein matrix (Pyler, 1988).

Effects of Rising Night-Time Temperatures on Wheat

Overall, rising temperatures reduce spikelet fertility, grains per spike, grain size, and quality. Reductions in yield and quality will cost growers. Most wheat growing regions of the world have been subjected to increases in both daytime and nighttime temperatures. Night time temperatures have been shown to be increasing three times more than daytime temperatures (Karl *et al.*, 1993; Easterling *et al.*, 1997). The extreme differential changes in temperature increments during day and night could play a pivotal role in wheat production (Lobell and Ortiz-Monasterio, 2007; Prasad *et al.*, 2008; Zhang *et al.*, 2013). However, most research on crop development and grain yield response to climate change has been founded on mean daytime air temperature. Therefore, understanding effects of elevated night-time temperature on winter wheat development and yield is essential for generating techniques to manage potential impacts of warming on agricultural systems worldwide (Zhang *et al.*, 2013). Many studies have found that yield and biomass production of cereal crops, such as rice and wheat, were affected by increased night-time temperatures associated with climate change. These studies found yield decreases in rice and wheat as night-time temperature increased (Cheng *et al.*, 2009; Mohammed and Tarpley, 2009, Lobell and Ortiz-Monasterio, 2007). For instance, winter wheat yields exhibited a 27% yield decrease when night-time temperature was increased by 2.58°C because high night-time temperature decreased tiller fertility, reducing the number of spikelets and grains per spike (Fang *et al.* 2010). Alternatively, some experiments have revealed that increased night-time temperatures had no affect on winter wheat yields (Fang *et al.*, 2012). In other instances, nocturnal warming was shown to have a positive effect on winter wheat yields. For example, one study observed a significant increase in winter wheat yields because night-time warming reduced tiller infertility. The differing results of wheat performance under elevated temperatures conveys the need for controlled daytime and nighttime warming experiments to assess the

warming response of winter wheat genotypes in different wheat growing regions to develop breeding strategies for future climates appropriate to each production area.

Effect of Warming on Plant NUE

Breeding for nitrogen-use efficiency is thought to be a possible strategy to develop wheat lines adapted to warmer environments. This is because these genotypes are able to take up more N and store it in their stem reserves. When the photosynthetic capacity of the plant is compromised due to elevated temperatures, the plant can utilize post-anthesis N stored stem reserves to continue grain filling and produce yield (Farooq *et al.*, 2011). Adaptation to heat stress has been shown to be related to the plants ability to accumulate stem reserves prior to anthesis (Blum *et al.*, 1994; Farooq *et al.*, 2011). There is evidence of genotypic variation for assimilate contribution to grain filling under heat stress (Yang *et al.*, 2002; Farooq *et al*, 2011). Variation in NUE in wheat has been documented and is likely to vary under elevated temperatures as well.

However, little has been explained in the literature on the effect warming has on NUE and plant N and how these traits are related to plant performance under warming (Ortiz-Monstarerio *et al.*, 1997; Foulkes *et al.* 1998; Brancourt-Hulmel *et al.*, 2003; Muurinen *et al.*, 2007; Barraclough *et al.*, 2010; Gaju et al, 2011). Warming does possibly enhance nitrogen (N) mineralization in the soil, increasing soil mineral N and resulting in increased N losses through leaching if N release is not synchronized with plant growth (Patil et al, 2012). These changes in soil N could cause changes in N uptake by plant roots, causing N uptake to decrease due to early maturation under warming (Sardans *et al.* 2008; Patil *et al.* 2010; Patil *et al.*, 2012). However, varieties with vast root systems may be more adapted to warmed environments. For example, studies on grass species have shown that root morphology impacts NUE by affecting NUpE (Louahlia *et al.*, 2000; Maire *et al.*, 2009; Miller *et al.*, 2007). Also, N utilization in above ground vegetative material and subsequent grain filling period would likely be affected by changed physiological processes resulting from rising temperature (Wolfe-Bellin *et al.* 2006; Prieto *et al.* 2009). Consequently, these physiological changes would have a profound impact on plant production (Li *et al.* 2011).

Several studies have examined the effects of increased temperature on N uptake and allocation in some crop species (Jonassona *et al.* 2004; An *et al.* 2005; Yang *et al.* 2011). For example, a night-time warming field experiment using reflective curtains showed that N accumulation in winter wheat during anthesis was 17-43% higher in the warmed than the unwarmed treatment. However, N utilization efficiency was decreased in the warmed treatment causing reduced N allocation towards yield during grain filling, resulting in a 6-25% yield decrease (Zhang *et al.* 2013). Total plant N content at anthesis and maturity, along with grain N content has been shown to decrease under dryer conditions due to a lower NUpE and subsequent NUtE, especially at higher N levels (Giuliani *et al.*, 2011). Therefore, researchers and breeders must continue to develop a better understanding of the fundamental mechanisms and traits associated with nitrogen uptake and utilization efficiency under warmed environments.

Chapter 3

Using High-throughput Phenotyping through Canopy Spectral Reflectance to Estimate Nitrogen Use Efficiency in the Soft Red Winter Wheat Elite Mapping Panel

Introduction

Nitrogen (N) is a critical nutrient for canopy growth, and canopy photosynthesis drives grain yield and grain quality (Hawkesford, 2012). However, crops do not utilize fertilizer N very efficiently. On average fertilizer N efficiency is less than 50% because the fertilizer N can be lost through leaching, dentrification, or volitization (Ladha *et al.*, 2005). Loss of N can have both economic and environmental costs to growers. For example, global fertilizer NUE for cereal production is around 33%, thus the N that was lost costs $15.9 billion (USD) (Raun *et al.*, 2001). Excess N has been shown to have adverse environmental impacts, such as eutrophication of freshwater and marine ecosystems that occurs when high quantities of N fertilizer are added to soil and then washed into the stream through runoff (Sieling et al, 2009). Therefore, great interest has been focused on crop varieties with high nitrogen use-efficiency (NUE) because these plants would be expected to minimize environmental and production costs associated with addition of excess N to agricultural systems. Increasing NUE can also reduce greenhouse gases emitted by the crop, associated with the production of grain yield (Gaju *et al.*, 2011). In wheat production, 70% of N fertilizer accounts for increased greenhouse gases mostly in the form of nitrous oxide (Mortimer *et al.*, 2004). Nitrous oxide is a powerful greenhouse gas that is released into the atmosphere after N fertilizer application (Bouwman *et al*., 2002). According to the Intergovernmental Panel on Climate Change report, developing crops with better NUE can shrink N_2O emissions and ultimately decrease green house gas emissions from N fertilizer products (IPCC, 2001). As a result, selecting and developing N use efficient crops has gained momentum among breeders.

Nitrogen use efficiency is defined as grain yield divided by the soil N supply (soil and fertilizer N) (Moll *et al.* 1982). In wheat, evidence has shown that yield gains have slowed since the 1990s (Muurinen *et al.*, 2007). *Triticuum aestivum* (winter wheat) is an important component of the national and global food supply. As human population

growth continues, worldwide demand for wheat will continue to increase (Ludwig and Asseng, 2006; Parry, 2012). Providing food for future populations while assuring sustainability of agricultural systems is a growing dilemma. To achieve this goal in agricultural production, breeders must overcome other obstacles such as climate change. Nitrogen-use efficient crop varieties may be a good option to ensure sustainability in agricultural systems and meet future consumer demands, especially when faced with a changing climate. However, NUE is a complex trait and requires labor intensive sampling to identify NUE superior lines (Ma *et al.*, 1996; Raun *et al.*, 2001; Crain *et al.*, 2012).

More rapid and efficient selection for high yielding and nitrogen use efficient plants may be possible through high throughput phenotyping. There have been great advances in genotyping technologies over the years (Winterhaltera *et al.*, 2010). However, deficiencies in phenotyping abilities reduce the capacity to accurately assess the genetics governing quantitative traits (Winterhaltera *et al.*, 2010; Montes *et al.*, 2011). Using high throughput phenotyping techniques can connect the genotype and phenotype. Canopy spectral reflectance (CSR) devices can be utilized to implement high throughput phenotyping for complex traits, such as nitrogen concentration, associated with NUE (Li et al, 2014). Canopy spectral refelctance devices measure the amount of light reflected/absorbed by the plant's canopy surface. Genotypic variation and environmental stress can affect the amount of light reflected. Thus, the use of CSR in selection for NUE may be a rapid and inexpensive option (Raun *et al.*, 2001).

Nitrogen use efficiency can be estimated through traditional soil sampling and plant tissue testing. However, traditional monitoring and evaluation of crop nitrogen is very destructive to plant samples, involve a rigorous work load, and compromise the accuracy of data results. Therefore, CSR techniques could be used for nondestructive and real-time monitoring of crop N status. Canopy sprectral reflectance indices, such as the normalized difference vegetative index (NDVI) or red infrared ratio (RIRR), have been shown to have high correlations with wheat grain yield, biomass and N concentration (Ma *et al.*, 1996; Raun *et al.*, 2001; Crain *et al.*, 2012). These indices are already being utilized to manage N in the field. For instance, in China, researchers are using CSR indices to estimate N uptake of winter wheat to better manage N fertilizer

application (Li *et al.*, 2012). The CSR estimates of biomass and N content can be used to estimate uptake and utilization efficiency. The relationship of CSR with biomass is of great interest, since biomass is an essential element related to NUE and yield (Crain *et al.*, 2012). Measuring N content through CSR techniques could also give insight into the plant's physiology and may indicate other traits to investigate that may benefit NUE, such as root physiology activity and N partitioning. For instance, it is reasonable to infer that plants with good NUE may have superior root structure that allows these plants to extract more N from the soil to be utilized in the plant (Li *et al.*, 2012; Li *et al.*, 2014). Therefore, 280 soft red winter wheat lines were grown in an augmented randomized complete block design in Lexington, KY to determine the utility of the CSR indices to identify N efficient winter wheat cultivars compared to traditional N sampling and identify traits associated with NUE through association mapping.

Materials and Methods

Site Description and Experimental Design

The 320 entry NAM soft winter wheat panel was grown for two years (2012 and 2013) in five 8x8 blocks as an unreplicated randomized augmented block design at University of Kentucky Spindletop Research Farm in Lexington, KY (38°7'37.81''N, 84°29 44.85'' W). Two-hundred and eighty soft red winter wheat genotypes were tested; the cultivar Branson was used as a check, eight replicates per block. Planting dates were 10 October 2011 and 18 October 2012. The experimental unit each year was a single 6-row yield plot 3.3 m in length, 1.2 m wide. The site was characterized by Maury silt loam [fine, mixed, semiactive, mesic Typic Paleudalfs] soil. 101 kg N $^{ha-1}$ was applied in a 34 kg N $^{ha-1}$ and 67 kg N $^{ha-1}$ split on 22 February and 9 March 2012. 101 kg N $^{ha-1}$ was applied in a 34 kg N $^{ha-1}$ and 67 kg N $^{ha-1}$ split, 14 March 2014 and 4 April 2013 respectively. A weather station was placed at the site to measure temperature data throughout the duration of the study.

Field Sampling and Data Collection

Canopy Spectral Reflectance

At Feekes 10 (booting stage), N status of the plant was measured using the JAZ instrument, a canopy spectral reflectance device (CSR). Canopy sprectral reflectance measurements taken at Feekes 10 have been shown to be highly correlated with CSR N indices (Pinter *et al.*, 1981; Raun *et al*., 2001). JAZ values for each plot were generated by using the continuous scanning method, in which the instrument is scanned along the center of the plot by holding the fibers level and moving the fibers over the plot center in a slow circular motion. This allows the entire plot to be integrated into one reading, as well as reducing noise seen at higher wavelengths. The scanning method gives repeatable results and has been shown to have a very strong r-square value of 0.99 between the different scans of the plots. The indices normalized differential vegetative index (NDVI) (780nm- 680nm/(780nm+680nm) and red infrared ratio (RIRR) (680nm/780nm) were calculated for each of the entries in each block from the JAZ data. Wavelength 780 nm represents spectral reflectance measurement from the near infrared spectrum. Wavelength 680 nm represents spectral reflectance measurement from the visible spectrum (red).

Agronomic Traits

For each plot, heading date was recorded when 50% of the spikes in a plot had emerged from the leaf sheath. Anthesis date was recorded in 2013 only when 50% of the spikes were flowering. Plot length and height of each plot were recorded at the soft dough stage. Percent of plants lodging in a plot were also recorded at the soft dough stage, but only for the 2013 field season; there was no lodging in 2012. The soft dough stage is equivalent to physiological maturity, which is when maximum dry matter accumulation has occurred and the kernel turns a buff color. At anthesis and harvest maturity (when the kernel was hard and could not be split by thumbnail), 10 non-damaged flag leaves were collected randomly down the length of each plot and air dried in the greenhouse. Leaves were ground to a fine powder using a UDY cyclone mill and ground samples were dried in an oven at 55^{o}C overnight. Twenty to 25 mg of ground sample was

weighed out for combustion analysis using a Flash EA1112 elemental analyzer to measure N concentration.

Field plots were combine harvested and grain was measured for yield. Grain test weight and moisture content were estimated using the GAC instrument. After harvest, cut straw was raked on the appropriate plot and biomass was measured in the field using a Hege forage harvester for both years. A 50g subsample of grain was collected from each plot to measure whole grain protein content by Near Infrared Reflectance (NIR) in 2013 only.

Statistical Analysis

Analysis of variance (ANOVA) was performed using the General Linear Models procedure (Proc GLM; SAS 2002) to determine effects of both years at Lexington, KY. The model used for the augmented randomized complete block design was:

$$Y_{ij} = \mu + B_j + T_i + E_{ij}$$

Where: Yij = the observation in the *i*th genotype in the *j*th block, μ = the overall mean, $T_i = C_i + X_i (C_i)$ = the $_i$th genotype effect (the effect of the $_i$th check and effect of the $_i$th genotype nested within the *i*th check), Eij = the residual error (Scott and Milliken, 1993). LSMEANS were computed to measure treatment differences among genotypes and years.

PROC CORR (SAS 2002) was used to analyze the relationship among traits on an entry mean basis.

Association Mapping in TASSEL

All entries in the mapping panel were genotyped with the 9K Illumina SNP chip to identify single nucleotide polymorphisms (SNP) associated with the traits measured during the course of the study. TASSEL (http://www.maizegenetics.net) software was used to carry out association mapping. The Q+K method was implemented as a mixed linear model to determine association of the N traits and agronomic traits measured with QTL markers. The statistical model used was described as:

$$Y= Xb+ Zu+e$$

where y is the vector of observations; b is an unknown vector containing fixed effects including genetic marker and population structure (Q); u is an unknown vector of random additive genetic effects from multiple background QTL for individuals or lines; X and Z are the known design matrices; and e is the unobserved vector of random residuals (Bradbury *et al.* 2007).

Results and Discussion

Description of Climate Conditions in 2012-2013

In 2012, there were unusually warm temperatures in March through May, which accelerated growth and reproductive development in the wheat crop. Average temperature during this time period was 16.1°C, average minimum was 10°C, and average maximum was 21.7°C, which was on average 6 degrees above the 30-year normal. The 2012 wheat crop in Kentucky headed about three to four weeks earlier than normal and was harvested approximately three weeks earlier than normal. Low temperatures in April caused freeze damage throughout the state of Kentucky in 2012. Total precipitation between March and May was 20.4 cm which fell considerably below the 30-year normal by 12 cm (http://wwwagwx.ca.uky.edu/). In 2013, temperatures between March and May were much lower than in 2012. Average temperature in 2013 was 12.2°C, average minimum temperature was 7.2°C, and average maximum temperature was 17.2°C. Total precipitation between March and May was 40.3 cm, which was 8 cm above the 30-year normal (http://wwwagwx.ca.uky.edu/). In 2013, there was residual N left over from the previous corn crop which on average caused 47% lodging among test plots, resulting in yield losses.

Agronomic Traits and N Traits

From the ANOVA, the variable C tests the hypothesis that the check means and experimental means are equal. The X(C) variable tests the hypothesis that all the means of the experiment are equal (Scott and Milliken, 1993). In 2012, all traits other than percent soil moisture were significantly different between checks and genotypes being tested and among genotypes being tested ($p<0.01$). In 2012, the test genotypes had

higher yield, biomass, test weight, later heading date, and were taller than the check (Table 3.1). In 2013, only test weight ($p<0.01$), heading date ($p<0.01$), height ($p<0.01$), and % lodging ($p<0.05$) were significantly different between checks and test genotypes. Yield ($p<0.05$), total biomass ($p<0.05$), test weight ($p<0.01$), heading date ($p<0.01$), anthesis date ($p<0.01$), height ($p<0.01$), and lodging ($p<0.01$) were significantly different among the test genotypes (Table 3.2). Agronomic traits were significantly different between each year (Table 3.3). The check genotype was not significantly different for agronomic traits between years. The test genotypes were significantly different between years for all agronomic traits. There was no check*year interaction for the agronomic traits. There was a genotype*year interaction for the test genotypes for agronomic traits yield, total biomass, heading date, and height (Table 3.3). Yield and total biomass were lower in 2012 than 2013. Heading date was earlier and plants were shorter in 2012 (Tables 3.1, 3.2, 3.3). The differences in heading date, height, and yield are likely due to the environmental differences experienced each year. In 2012, plants were exposed to higher temperatures than in 2013. Elevated temperatures caused plants in 2012 to develop earlier, possibly shortening the grain filling period, thus reducing yield. Differences in height and biomass may have also been caused by more rapid development in 2012 than in 2013. However, in 2013, residual N was left over from the previous crop causing biomass, plant height, and lodging to be much higher than in 2012. Genotypic variation for all agronomic traits was higher in 2012 than in 2013 (Table 3.1 and 3.2). The 2013 field season had much more favorable growing conditions than 2012, thus the true variation among the lines was masked. The 2012 environment may have provided better conditions for selection of high performance varieties rather than 2013.

There was no significant difference in flag leaf N at anthesis between checks and test genotypes or among genotypes in either year. There was a significant difference between genotypes being tested for flag leaf N concentration at maturity ($p<0.05$) in the 2012 environment, but not in 2013 (Table 3.4 and 3.5). There was significant difference between test genotypes for NUE in both years ($p<0.01$ 2012; $p<0.05$ 2013). Percent flag leaf N at anthesis and maturity was significantly different between years, $p<0.05$ and $p<0.01$ respectively (Table 3.6). N concentration at anthesis was 4.2% in 2012 and 4.0% in 2013. Nitrogen concentration at maturity was 2.5 % in 2012 and 1.2% in 2013.

Nitrogen concentration at anthesis between the years was similar. However, N concentration at maturity was higher than in 2012 than 2013 (Table 3.6). This is likely related to the accelerated development experienced due to the higher than normal temperatures experienced under 2012 field conditions. Due to quickened development there was likely less time to remobilize N to the grain, causing more N to be left over in the vegetative tissue at maturity in 2012 than 2013, which experienced more favorable field conditions resulting in a longer grain filling period and more N being remobilized to the grain.

There was a significant negative relationship between flag leaf N concentration at maturity and yield ($p<0.01$; Table 3.7). This may be an indicator of N remobilization among some of the genotypes. Winter wheat varieties that exhibit N remobilization may be more adapted to low N environments. Therefore, selecting wheat varieties with high N remobilization would allow plants to improve nutrient economy and to survive in high stress environments, like in 2012, which experienced unusually warm temperatures between March and May. Through N recycling, wheat varieties would be able to better support the development of younger leaves and increase yield under nutrient-limiting conditions (Masclaux-Daubresse *et al.*, 2010). In 2013, there was also a negative relationship between yield and N concentration at maturity (Table 3.7). However, the relationship was very weak. This was likely caused by the residual N left over from the previous crop, causing increased biomass and loss of yield due to lodging, thus possibly weakening the correlation between yield and N concentration in the flag leaf at maturity.

Utility of Canopy Spectral Reflectance devices for High-throughput Phenotyping of NUE

Canopy Spectral reflectance measurements were taken at the booting stage, considered to be the proper stage to estimate NUE using the CSR indices. The rationale is that at this stage N is most efficiently utilized for grain production and therefore would show the best relationship to the N related NDVI and RIRR indices (Raun *et al.,* 2001). In 2012, NDVI and RIRR indices were significantly different between checks and genotypes being tested and among genotypes being tested (Table 3.4). In 2013, no significant variation was seen between checks and genotypes being tested and among genotypes for NDVI or RIRR (Table 3.5). In 2012, temperatures were much higher than

normal in the spring and summer months than in 2013. In 2012, plants were likely more stressed than in 2013, thus resulting in decreases for N traits and CSR indices since plants were exposed to warmer temperatures. In both years, the CSR indices were significantly correlated to the agronomic traits ($P<0.01$) (Table 3.7). The NDVI index was positively correlated to yield, straw, biomass, heading date, and height and harvest index. In 2013, however, NDVI had a significant negative correlation to HI. This is likely due to the large amount of lodging in 2013, whereas in 2012 lodging was negligible. Canopy spectral reflectance indices were not significantly correlated to the flag leaf N content at anthesis or maturity (Table 3.7). As observed in previous studies, biomass and grain yield from the field study were significantly correlated to the CSR indices (Ma *et al.*, 1996; Raun *et al.*, 2001; Crain *et al.*, 2012). Because other agronomic traits like HI, height, and heading date are related to yield; it is not a surprise that they were significantly correlated to the CSR indices as well. But unlike previous studies, the N concentration data was not strongly correlated to the CSR indices (Table 3.7). Even though the correlations between agronomic traits and NDVI were significant, the actual correlations were weak, and therefore no strong correlation between the measured traits and CSR indices was observed. The lack of strong correlations with the CSR indices and the traits investigated is possibly due to the use of a passive CSR device over an active one. The performance of the JAZ instrument is dependent on the weather and the amount of sunlight available to produce good readings. Kentucky's environment is very variable. Cloudy or windy weather, which was common during the window in which the nitrogen CSR indice readings could be taken (Feekes 10, booting), can disrupt CSR readings taken by the JAZ, causing the N indices calculated (NDVI and RIRR) from the JAZ to be inaccurate.

Association Mapping of Agronomic and N Traits

In 2012, there were no marker trait associations that were significant at 0.05 or 0.01 after the Bonferroni multiple test correction (0.05/21067; 0.01/21067), $2.4*10^{-6}$; $4.7*10^{-7}$ (Table 3.8). In 2013, several SNP's were associated with grain protein ($p<4.7*10^{-7}$) and heading date ($p<2.4*10^{-6}$), but there were no associations with the N traits measured (Table 3.9). The numerator is the *p*-value being tested and the

denominator in the Bonferroni correction is the number of SNPs being tested. The Bonferroni correction adjusts *p* values when multiple dependent or independent statistical tests are being performed concurrently on a single data set. The Bonferroni correction is performed by dividing the critical *p* value (0.05, 0.01) by the number of comparisons being made, in this case the number of SNP's (21067). The modified *p* value ($2.4*10^{-6}$; $4.7*10^{-7}$) is then used to test the statistical power of the data (Bradbury *et al.*, 2007). Even though some associations were found in traits like grain protein and heading date, these traits have been heavily studied and QTL's have been identified that are associated with these traits (Bogard *et al.*, 2014; Sun *et al.*, 2008). Using a larger number of genotypes and growing the lines in low input systems under multiple environments, may increase the success of finding QTL's associated with NUE.

Table 3.1. LSMEANS for agronomic traits measured for 280 genotypes being tested (tests) and the check, genotype Branson replicated, generated from the ANOVA Lexington, KY elite TCAP mapping panel 2012. Block (B), C (check), X(C) (genotype). Yield (Y) (kg ha^{-1}), vegetative biomass (kg ha^{-1}) (Vb), total biomass (kg ha^{-1}) (TB), harvest index (HI) (%), test weight (Twt) (kg hL^{-1}), moisture (M) (%), heading date (Hd) (May 1=1, May 2=2, etc.), height (H) (cm), lodging (%).

	Y	Vb	TB	HI	Twt	M	Hd	H	Lg
Tests	2321.2	2787.2	5108.4	53.1	73.4	13.4	25	76.6	0
Check	2190.4	2141.0	4331.4	58.6	70.9	13.5	22	71.4	0
B	0.12	0.011	0.011	0.06	0.18	<0.0001	0.15	0.89	.
C	0.05	<0.0001	<0.0001	<0.0001	<0.0001	0.26	<0.0001	<0.0001	.
X(C)	0.001	0.001	0.003	0.0004	<0.0001	0.17	<0.0001	0.013	.

Table 3.2. LSMEANS for agronomic traits measured for 280 genotypes being tested (tests) and the check, genotype Branson replicated, generated from the ANOVA Lexington, KY elite TCAP mapping panel 2013. Block (B), C (check), X(C) (genotype). Yield (Y) (kg ha^{-1}), vegetative biomass (kg ha^{-1}) (Vb), total biomass (kg ha^{-1}) (TB), harvest index (HI) (%), test weight (Twt) (kg hL^{-1}), moisture (M) (%), heading date (Hd) (May 1=1, May 2=2, etc.), anthesis date (Ad) (May 1=1, May 2=2, etc.), height (H) (cm), lodging (%).

	Y	Vb	TB	HI	Twt	M	Hd	Ad	H	Lg
Tests	2383.5	6132.4	8515.8	37.3	73.2	16.2	11	16	97.1	47.0
Check	2541.8	6027.8	8569.6	39.9	71.2	16.3	10	15	93.9	38.4
B	0.63	0.56	0.41	0.51	0.05	0.15	0.35	0.50	0.17	<0.0001
C	0.15	0.51	0.15	0.38	<0.0001	0.49	<0.0001	0.06	0.003	0.05
X(C)	0.014	0.18	0.03	0.32	0.006	0.17	<0.0001	0.009	<0.0001	0.001

Table 3.3. LSMEANS for agronomic traits measured for 280 genotypes being tested (tests) and the check, genotype Branson replicated, generated from the ANOVA Lexington, KY elite TCAP mapping panel 2012-2013. Year (Yr), block (B), C (check), X (genotypes being tested). Yield (Y) (kg ha^{-1}), vegetative biomass (kg ha^{-1}) (Vb), total biomass (kg ha^{-1}) (TB), harvest index (HI) (%), test weight (Twt)(kg hL^{-1}), moisture (M) (%), heading date (Hd) (May 1=1, May 2=2, etc.), height (H) (cm), lodging (%).

Yr		Y	Vb	TB	HI	Twt	M	Hd	H
2012	Tests	2321.2	2787.2	5108.4	53.1	73.4	13.4	25	76.6
	Check	2190.4	2141.0	4331.4	58.6	70.9	13.5	22	71.4
2013	Tests	2383.5	6132.4	8515.8	37.3	73.2	16.2	11	97.1
	Check	2541.8	6027.8	8569.6	39.9	71.2	16.3	10	93.9
	Yr	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	B(Yr)	0.50	0.42	0.14	0.34	0.002	<0.0001	0.23	0.47
	X	<0.0001	0.03	0.001	0.015	<0.0001	0.0223	<0.0001	<0.0001
	C	0.28	0.55	0.23	0.78	0.48	0.14	0.96	0.63
	Yr*X	0.002	0.06	0.004	0.07	0.06	0.28	<0.0001	0.02
	Yr*C	0.36	0.27	0.06	0.38	0.67	0.98	0.32	0.39

Table 3.4. LSMEANS for N traits measured for 280 genotypes being tested (tests) and the check, genotype Branson replicated, generated from the ANOVA Lexington, KY elite TCAP mapping panel 2012. Block (B), C (check), X(C) (genotype). Red visible spectrum (R) (%), near-infrared spectrum (NIR) (%), Normalized difference vegetative index (NDVI), red infrared ratio (RIRR), % N at anthesis in flag leaf (Na), % N at maturity in flag leaf (Nm), nitrogen-use efficiency (NUE).

	R	NIR	NDVI	RIRR	Na	Nm	NUE
Tests	3.8	43.2	0.84	0.09	4.2	2.5	23.0
Check	4.7	40.8	0.80	0.11	4.2	2.6	21.7
B	0.0001	<0.0001	0.07	0.06	0.84	0.37	0.12
C	<0.0001	0.09	<0.0001	<0.0001	0.75	0.68	0.051
X(C)	<0.0001	0.0003	0.0004	<0.0001	0.15	0.019	0.001

Table 3.5. LSMEANS for N traits measured for 280 genotypes being tested (tests) and the check, genotype Branson replicated, generated from the ANOVA Lexington, KY elite TCAP mapping panel 2013. Block (B), C (check), X(C) (genotype). Protein (P) (%), Red visible spectrum (R) (%), near-infrared spectrum (NIR) (%), Normalized difference vegetative index (NDVI), red infrared ratio (RIRR), % N at anthesis in flag leaf (Na), % N at maturity in flag leaf (Nm), nitrogen-use efficiency (NUE).

	P	R670	NIR780	NDVI	RIRR	Na	Nm	NUE
Tests	10.8	1.6	49.2	0.94	0.03	4.0	1.2	23.6
Check	10.8	1.5	49.5	0.94	0.03	4.1	1.2	25.2
B	0.79	<0.0001	<0.0001	<0.0001	<0.0001	0.09	0.28	0.63
C	0.97	0.13	0.73	0.18	0.18	0.24	.11	0.15
X(C)	0.98	0.002	0.012	0.22	0.22	0.55	.43	0.014

Table 3.6. LSMEANS for N traits measured for 280 genotypes being tested (tests) and the check, genotype Branson replicated, generated from the ANOVA Lexington, KY elite TCAP mapping panel 2012-2013. Year (Yr), block (B), C (check), X (genotypes being tested). Red visible spectrum (R) (%), near-infrared spectrum (NIR) (%), Normalized difference vegetative index (NDVI), red infrared ratio (RIRR), % N at anthesis in flag leaf (Na), % N at maturity in flag leaf (Nm), nitrogen-use efficiency (NUE).

Yr		R670	NIR780	NDVI	RIRR	Na	Nm	NUE
2012	Tests	3.8	43.2	0.84	0.09	4.2	2.5	23.0
	Check	4.7	40.8	0.80	0.11	4.2	2.6	21.7
2013	Tests	1.6	49.2	0.94	0.03	4.0	1.2	23.6
	Check	1.5	49.5	0.94	0.03	4.1	1.2	25.2
	Yr	<0.0001	<0.0001	<0.0001	<0.0001	0.02	<0.0001	<0.0001
	B(Yr)	<0.0001	<0.0001	0.005	0.005	0.18	0.32	0.50
	X	<0.0001	<0.0001	<0.0001	<0.0001	0.16	0.004	<0.0001
	C	0.06	0.57	0.04	0.02	0.31	0.61	0.28
	Yr*X	<0.0001	0.0002	<0.0001	<0.0001	0.46	0.02	0.002
	Yr*C	0.03	0.79	0.09	0.06	0.39	0.28	0.36

Table 3.7. Pearson correlation coefficients for agronomic and N traits measured from the 56 soft red winter wheat lines grown each year elite TCAP mapping panel 2012-2013 Lexington, KY. 2012 correlations depicted above the diagonal; 2013 depicted below the diagonal. Yield (Y), heading date (Hd), anthesis date (Ad), height (H), vegetative biomass (Vb), total biomass (TB), test weight (Twt), harvest index (HI), % grain protein (Gp), % N anthesis in flag leaf (Na), % N maturity in flag leaf (Nm), Red visible spectrum (R), near infrared spectrum (NIR), normalized difference vegetative index (NDVI), red infrared ratio (RIRR), % lodged (Lg), nitrogen use efficiency (NUE).

	Y	Vb	TB	HI	R	NIR	NDVI	RIRR	Na	Nm	P	Twt	Hd	Ad	H	Lg	NUE
Y	.	0.26**	0.72**	0.41**	-0.18**	0.08	0.22**	-0.20	-0.03	-0.30**	.	0.36**	-0.04	.	0.04	.	1.00**
Vb	-0.17**	.	0.84**	-0.75**	-0.17	0.31**	0.33**	-0.26*	-0.08	-0.06	.	0.24**	0.25**	.	0.44**	.	0.26**
TB	0.39**	0.83**	.	-0.29**	-0.22**	0.25**	0.36**	-0.29**	-0.08	-0.18	.	0.33**	0.14	.	0.33**	.	0.72**
HI	0.72**	-0.77**	-0.30**	.	0.03	-0.24**	-0.16	0.11	0.05	-0.11	.	-0.02	-0.28**	.	-0.39**	.	0.41**
R	-0.02	0.00	-0.03	-0.05	.	0.13	-0.90**	0.93**	-0.02	-0.06	.	-0.08	-0.29**	.	-0.22**	.	-0.18**
NIR	-0.16**	0.02	-0.09	-0.14*	0.72**	.	0.26**	-0.22**	0.03	-0.19**	.	0.09	0.08	.	0.09	.	0.08
NDVI	-0.16**	0.04	-0.05	-0.11*	-0.66**	0.02	.	-0.97**	0.04	0.01	.	0.11	0.36**	.	0.30**	.	0.22**
RIRR	0.16**	-0.04	0.05	0.11*	0.66**	-0.02	-0.99**	.	-0.04	0.02	.	-0.12	-0.31**	.	-0.23**	.	-0.20**
Na	0.04	0.17**	0.18**	-0.08	-0.09	-0.08	0.06	-0.06	.	0.13*	.	0.01	-0.14*	.	-0.05	.	-0.03
Nm	-0.07	0.14*	0.08	-0.14*	-0.02	0.06	0.12*	-0.12*	0.03	.	.	-0.37**	0.02	.	0.07	.	-0.30**
P	-0.01	0.01	-0.01	-0.02	0.17**	0.13*	-0.08	0.08	0.06	0.13*	.	.	.	.	.	.	.
Twt	0.13*	-0.01	0.04	0.07	0.05	-0.10	-0.17**	0.17**	0.04	-0.11	0.01	.	0.04	.	0.22**	.	0.36**
Hd	-0.06	0.01	-0.03	-0.06	-0.13*	-0.15**	0.03	-0.03	0.05	-0.10	-0.07	0.16**	.	.	0.30**	.	-0.04
Ad	-0.05	0.00	-0.03	-0.04	-0.12*	-0.10	0.07	-0.07	0.05	-0.03	-0.07	0.09	0.77**	.	.	.	.
H	-0.11	0.06	-0.01	-0.12*	-0.08	-0.07	0.08	-0.08	0.03	-0.05	-0.05	0.28**	0.33**	0.33**	.	.	0.04
Lg	-0.61**	0.08	-0.28**	-0.46**	-0.01	0.20**	0.27**	-0.27**	-0.04	0.09	-0.01	-0.08	0.06	0.07	0.20**	.	.
NUE	1.00**	-0.17**	0.39**	0.72**	-0.02	-0.16**	-0.16**	0.16**	0.04	-0.07	-0.01	0.13*	-0.06	-0.05	-0.11*	-0.61**	.

*p<0.05, **p<0.01

Table 3.8. Association mapping of measured traits and markers using the Mixed Linear Model in TASSEL of the soft red winter elite mapping panel 2012 Lexington, KY. Bonferroni test correction was used to identify significant markers. Heading date (Hd), height (H), nitrogen use efficiency (NUE), yield (Y).

Trait	Marker	Locus	Site	F	p	Marker R^2
Hd	Excalibur_c22419_460	7D	9304	17.4	4.18E-05	0.065
Hd	GENE-3129_890	7D	9122	16.2	7.59E-05	0.060
Hd	IWA1681	5D	11663	16.1	7.86E-05	0.060
H	Excalibur_c7338_242	7B	13359	11.1	2.39E-05	0.082
H	Kukri_c16814_103	7B	13359	11.0	2.53E-05	0.081
H	TA003945-0379	---	---	11.0	2.68E-05	0.081
H	Excalibur_c7338_563	7B	13359	10.9	2.82E-05	0.081
H	Kukri_c40909_784	3B	6572	10.7	3.32E-05	0.079
H	RAC875_c37934_285	1A	4845	10.5	3.95E-05	0.078
H	IWA5788	3B	6555	10.4	4.35E-05	0.077
H	IWA5813	3B	6555	10.4	4.35E-05	0.077
H	RFL_Contig3799_987	3B	6572	10.4	4.35E-05	0.077
H	Excalibur_c15744_322	6B	37	10.4	4.49E-05	0.080
H	BS00023203_51	1A	10872	10.1	5.69E-05	0.075
H	tplb0059d21_1032	1A	10872	10.2	5.83E-05	0.079
H	IACX8060	2A	6565	10.1	6.16E-05	0.075
H	BS00070797_51	2A	6565	10.0	6.65E-05	0.074
H	Excalibur_rep_c67994_169	2A	6565	10.0	6.65E-05	0.074
H	GENE-0749_215	2A	6565	10.0	6.65E-05	0.074
H	IWA2059	2A	6565	10.0	6.65E-05	0.074
H	IWA2696	2A	6565	10.0	6.65E-05	0.074
H	Kukri_rep_c104307_905	2A	6565	10.0	6.65E-05	0.074
H	RAC875_rep_c78518_198	2A	6565	10.0	6.65E-05	0.074
H	IWA7168	4B	7146	9.7	8.32E-05	0.072
H	BS00022522_51	7B	13359	9.6	9.92E-05	0.071
H	BS00083578_51	7B	13359	9.6	9.92E-05	0.071
H	CAP12_rep_c7901_114	3B	6636	9.5	9.99E-05	0.071
NUE	IACX7789	---	---	16.8	5.49E-05	0.063
NUE	Kukri_c13463_728	---	---	16.8	5.49E-05	0.063
Y	IACX7789	---	---	17.5	3.88E-05	0.066
Y	Kukri_c13463_728	---	---	17.5	3.88E-05	0.066
Y	RAC875_c32826_485	4B	7946	15.7	9.81E-05	0.059
Y	Excalibur_c24614_1203	4B	7946	15.6	9.89E-05	0.059

**$p<4.7*10^{-7}$; *$p<2.4*10^{-6}$

Table 3.9. Association mapping of measured traits and markers using the Mixed Linear Model in TASSEL of the soft red winter elite mapping panel 2013 Lexington, KY. Bonferroni test correction was used to identify significant markers. Grain protein (Gp) and heading date (Hd).

Trait	Marker	Locus	Site	F	p	MarkerR^2
Gp	BS00023080_51	6B	9301	17.2	1.00E-07**	0.129
Gp	BS00109717_51	6B	9301	17.2	1.00E-07**	0.129
Gp	IAAV1218	6B	9301	17.2	1.00E-07**	0.129
Gp	Tdurum_contig17421_310	6B	9301	17.2	1.00E-07**	0.129
Gp	BS00064423_51	5B	16132	17.1	1.06E-07**	0.129
Gp	RAC875_c82640_416	6B	9301	18.0	4.9E-08**	0.135
Gp	BobWhite_c3506_1151	6B	11045	17.3	8.5E-08**	0.132
Gp	Kukri_c16404_100	6B	9426	17.3	9.2E-08**	0.130
Gp	IWA3464	---	---	17.2	9.7E-08**	0.129
Hd	IACX9023	5A	8956	12.8	4.84E-06	0.097
Hd	Tdurum_contig55097_601	5A	8956	24.7	1.2E-.06*	0.093
Hd	BS00096940_51	---	---	14.2	1.4E-06*	0.109

**$p<4.7*10^{-7}$; *$p<2.4*10^{-6}$

Chapter 4

Effects of Warming on Nitrogen Use Efficiency at Different N Rates in Soft Red Winter Wheat

Introduction

Heat stress caused by climate change is projected to cause decreased yield and grain quality of winter wheat (*Triticum aestivum L.*) (Asseng ets al., 2014). With decreasing wheat yields and increasing worldwide population, growers ability to keep up with global demand may become more difficult. Both components of yield, grain number and grain weight, are susceptible to increased temperature (Ferris *et al.*, 1998). The developmental stage at which elevated temperatures occur will determine which component of grain yield will be affected. For example, during anthesis, temperatures above 20^0C may considerably decrease grain number per spike (Saini and Aspinall, 1982). Heat stress accelerates development of the spike, reducing spikelet number, resulting in fewer grains per spike (Saini and Aspinall, 1982; Porter and Gawith, 1999; Farooq *et al.*, 2011). Grain number can also be reduced during floral initiation. For instance, for every one °C increment temperature increase above optimum temperature during the 30 days preceding anthesis, grain number per spike decreased by 4% (Fischer, 1985; Wardlaw *et al.* 1989; Ottman *et al.*, 2012). However, these studies were conducted under controlled conditions and may not be comparable to field conditions. Controlled studies may also underestimate or overestimate the yield decreases from elevated temperatures that occur under field conditions. Wheat planted at different dates during the year can allow the plants to be exposed to diverse temperatures during the crop's development, which can give an indication of crop response to temperature under field conditions (Ottman *et al.*, 2012). For example, a comprehensive experiment conducted in India studying wheat response to temperature using staggered planting dates found that yield traits were negatively related to mean temperature 20 days before heading to 10 days after heading and 10 to 40 days after heading (Ortiz-Monasterio *et al.*, 1994). There are several examples in the literature in which late planting, causing plants to be exposed

to elevated temperatures, resulted in lower yields (Ali *et al.*, 2010; Musick and Dusek, 1980).

Nitrogen is a critical nutrient for wheat and other plants because it influences yield and grain quality (Hawkesford, 2012). Increasing nitrogen use efficiency (NUE) in winter wheat could reduce the negative effects of increased temperature caused by climate change because these genotypes are able to take up more N and store N in their stem reserves. When the photosynthetic capacity of the plant is compromised due to elevated temperatures, the plant can utilize N stored in stem reserves to continue grain filling and produce yield (Farooq *et al.*, 2011). Adaptation to heat stress has been shown to be related to the plants ability to accumulate N in stem reserves prior to anthesis (Blum *et al.*, 1994; Farooq *et al.*, 2011). There is evidence of genotypic variation for C and N assimilate contribution to grain filling under heat stress (Yang *et al.*, 2002; Farooq *et al*, 2011). Variation in NUE in wheat has been documented and is likely to vary under elevated temperatures as well. However, there has been little research done on NUE adaptation to heat stress caused by climate change, especially in looking at post-anthesis nitrogen uptake and root phenology. The high NUE genotypes identified could be incorporated into breeding programs to create a heat stress resistant cultivar. As a result, these cultivars could help wheat production remain stable or increase in environments with increasing temperatures. High NUE, temperature-tolerant genotypes may allow farmers to reduce their use of N fertilizer. This could decrease the economic and environmental costs caused by applying additional nitrogen to the soil. Additional research on developing heat stress tolerant crops could help ensure food security worldwide.

To study wheat N uptake and utilization response to temperature, a replicated hill plot preliminary study was planted in Lexington, KY under three N environments (0 kg N ha^{-1}, 101 kg N ha^{-1}, 168 kg N ha^{-1}). The experimental material comprised eight genotypes. Staggered planting dates (September, October, November) of the wheat genotypes simulated temperature increases caused by climate change. The objectives of this study were to: 1) identify genotypes that utilize nitrogen most efficiently when exposed to increased temperatures in high, moderate, and low N environments; 2)

determine which varieties best utilize nitrogen under heat stress; and 3) determine if root biomass contributes to NUE differences among genotypes.

Material and Methods

Site Description and Experimental Design

The study took place at University of Kentucky Spindletop Research Farm in Lexington, KY (38°7'37.81''N, 84°29 44.85'' W) (37°6'7.37'' N, 87°52'13.62''W). The site was characterized by Maury silt loam [fine, mixed, semiactive, mesic Typic Paleudalfs] soil. The preliminary study using replicated hill plots was planted as .23 m^2 plots under 3 N environments: low (0 kg N ha^{-1}), normal conditions (101 kg N ha^{-1}), and high (168 kg N ha^{-1}) N. Nitrogen fertilizer was applied as urea in a 34 kg N ha^{-1} and 67 kg N ha^{-1} split for the 101 kg ha^{-1} environment and a 56 kg N ha^{-1} and 112 kg N ha^{-1}split for the 168 kg N ha^{-1} environment. The 1st N application occurred on 14 March 2013 and the 2nd on 4 April 2013. To simulate the increased temperatures associated with climate change, the planting dates were staggered: 27 September, 25 October, and 29 November 2012. A Watch dog weather station was placed at the site to record temperature throughout the duration of the study.

Eight genotypes comprised the experimental material: Pembroke, Truman, Shirley, 25R32, KY97C-1238-17-1, KY02C-1058-03, KY04C-1128-38-1-5, and KY05C-1617-17-17-3. These genotypes differed in several traits such as heading date and height, though prior to the study genotypic differences in grain protein content and vegetative N content were unknown. Each N environment contained three replicates for each genotype/planting date combination. Within each plot, two hills were planted, one planted to be harvested at anthesis and one planted to be harvested at harvest maturity.

Field Sampling and Data Collection

Pre-N Soil Sampling

Prior to N application three soil cores were taken at a depth of 30.48 cm using 1.6 cm diameter soil probe, mixed by hand in a plastic bucket, air dried on paper bag, placed in a

paper soil bag, and dried at 55°C in an oven. Next, total N concentration of each sample was determined by combustion using the Flash EA 1112, 50-55 mg per sample. Average soil N across environments was 0.23%. Percent soil N was converted to lb ac^{-1} by multiplying % soil N by 20,000, assuming 2 million ppm of soil per acre (Havlin *et al*., 2014). Therefore, total N was 4,750 lb ac^{-1} or 5,320 kg ha^{-1}. However, a very small percent of total soil N is actually available to plants, between 1-2% (Havlin *et al*., 2014). Therefore, total plant N was multiplied by 0.01 to give a rough estimate of available soil N, 47.5 lb ac^{-1} or 53.2 kg ha^{-1}.

Resin Bag Sampling

Nylon resin bags filled with 5 g of mixed bed resin beads were placed 10 cm below each of the three replication of the two cultivars Pembroke and Truman in each N environment and planting date to measure N availability differences. These varieties differ greatly in heading date and height and thus were speculated to differ in development and N uptake. Resin bags were changed every two weeks starting in March until plants reached anthesis. When resin bags were removed and replaced, the bags were always transported to and from the field in an ice filled cooler.

Agronomic Traits

For each plot, heading date and anthesis date was recorded. Heading date was determined when 50% of the spikes had emerged in a hill plot. Anthesis date was recorded when 50% of the plants were flowering in a hill plot. Phyllochron measurements (Klepper *et al*, 1983) were collected to estimate developmental differences between each treatment and planting date. Measurements were taken by counting the number of leaves as they appeared on the main stem. Phyllochron measurements were collected every two days from Pembroke and Truman within each N environment and planting date until the emergence of the flag leaf.

Whole plant samples were hand harvested at anthesis and harvest maturity using a sickle, and were then air dried in the greenhouse. Harvest maturity was determined when the grain was hard and could not be split by thumbnail. Biomass was estimated from dried whole plant vegetative aboveground material harvested at anthesis and

maturity. Grain was mechanically threshed, and weighed to estimate yield. Aboveground material, which included leaves, stem, and grain was ground to a fine powder using the UDY cyclone mill. Ground grain and vegetative samples were dried in an oven at 55°C overnight and analyzed by combustion using the FlashEA 1112 analyzer to measure % N concentration, 20-25 mg per sample.

Data Processing and Analysis

Resin Bag Analysis

Ammonium and nitrate were extracted from the resin bags using the KCl extraction method (Giblin *et al*., 1994). Resin bags were shaken on a reciprocal shaker in 40 mL of KCl for one hour. The extraction liquid was then filtered through funnels lined with filter paper that had been pre-wetted with deionized water and 1 ml of each sample was pippetted into cluster tubes in a cluster box. The cluster tube box with samples was centrifuged for 27 minutes, after which, 15 µL of standards and samples was pippetted into the wells of two microplates, one for nitrate analysis and one for ammonium analysis.

To prepare the nitrate microplate for analysis, 200 µL of pH 8.5 ammonium buffer was added to each nitrate microplate well. A copperized cadmium reductor was placed into each well of the microplate and shaken for 60 minutes on a titer plate shaker to convert nitrate to nitrite (Nydhal, 1976; Crutchfield and Grove, 2011). The Griess reaction was used to colormetrically measure the nitrite concentration within each sample. To induce the Griess reaction, 60 µL of Griess reagent (4 mL of 1.0 % sulfanilamide in 3 N hydrochloric acid and 4 mL of 0.1 % N-(1-Naphthyl)) was added to each well of the microplate (Griess, 1858; Crutchfield and Grove, 2011). The microplate was shaken for additional 5 minutes. The microplate was inserted into the Microplate Versa Max Analyzer and nitrite levels within each well were read at 542nm (Henriksen and Selmer-Olsen, 1970; Crutchfield and Grove, 2011).

The ammonium microplate was colormetrically measured using a modified Berthelot reaction. The Berthelot reaction was induced by inserting 100 µL of sodium hydroxide-hypochlorite and 100 µL of phenol-nitroprusside into each microplate well

(Berthelot, 1859; Chaney and Marbach, 1962; Weatherburn, 1967; Ngo *et al.*, 1982). Then the microplate was shaken on a titer plate shaker for 45 minutes. Afterwards, the microplate was inserted into the Microplate Versa Max Analyzer and ammonium levels were measured at 630nm.

Measuring N Traits

From the combustion and soil N data, the N traits were calculated. Total plant N uptake was determined by summing plant N in grain (yield*%N) (kg ha^{-1}) and vegetative tissue at maturity (biomass *% vegetative N at maturity) (kg ha^{-1}). Post-anthesis N uptake was calculated by subtracting the N in vegetative tissue at anthesis (anthesis biomass*% vegetative N at anthesis) (kg ha^{-1}) by total plant N. Nitrogen-use efficiency (NUE) and NUE components (nitrogen uptake efficiency (NUpE) and nitrogen utilization efficiency (NUtE)) calculated using the following formulas: NUpE= Total plant N/ soil N (Pre-N soil N and fertilizer N), NUtE =yield/total plant N, NUE= (NUpE) (NUtE).

Root Traits

Root samples were excavated with a trowel at harvest maturity at a 10 cm depth (where main root mass is located in wheat (Lynch *et al*., 2014)) in each replication of the two genotypes Pembroke and Truman. Loose soil was gently shaken off the root samples and samples were then frozen for future analysis. Roots were thawed prior to analysis and were washed with cold deionized water to remove remaining soil and retain very small root parts through a 53 μm sieve. Root samples were dried in an oven at 55°C for 72 hrs and root mass was recorded.

Statistical Analysis

An analysis of variance (ANOVA) using the General Linear Model procedure (Proc GLM; SAS 2002) was used to determine treatment and varietal differences among the measured traits within each environment using the following model:

$$Y_{ijkl} = \mu + ENV_i + R(ENV)_{j(i)} + PLD_k + G_l + ENV*PLD_{ik} + ENV*G_{il} + G*PLD_{kl} \; ENV*PLD*G_{jikl} + E_{ijkl}$$

Where: Yijlk = the observation in the *l*th genotype in the *j*th rep in the *i*th environment and *k*th planting date, μ = the overall mean, R(ENV)j(i) = the effect of *j*th rep within *i*th environment, ENV*PLD$_{ik}$=the interaction effect of the *i*th environment with the *k*th planting date, ENV * G$_{il}$ = the effect of the interaction of the *i*th environment with the *l*th genotype, PLD*G$_{kl}$= the effect of the interaction of the *k*th planting date with the *l*th genotype, ENV*PLD*G$_{ijkl}$= the effect of the interaction of the *i*th environment and *k*th planting date with the *l*th genotype, E$_{ijkl}$ = the residual error.

PROC CORR procedure in SAS was used to estimate the correlation between N traits, agronomic traits, root biomass, N environment, planting date, and temperature on an entry mean basis (SAS 2002).

Results and Discussion

Developmental differences of winter wheat between planting date and N environment

Phyllochron is the time interval between the appearance of successive leaves on the main stem and can be used to describe development (Klepper *et al*, 1983). Phyllochron measurements were recorded daily until the flag leaf appeared (final leaf (leaf 6) prior to beginning of heading) on Pembroke and Truman and compared against accumulated growing degree days (Table 4.1). Development was quickened under the low N environment compared to the moderate and high N environments, as expected for each planting date for both Pembroke and Truman, shown by reduced duration of leaf appearance over time (Table 4.1). Genotypes planted later under each N environment developed more quickly than plants planted in September because later planting dates were exposed to higher temperatures during growth. The plants from the November planting were exposed to the highest temperatures and exhibited the most accelerated phyllochron rate, taking fewer growing degree days (GDD) for the subsequent leaves to emerge (Tables 4.8). Plants grown under low N environments and planted in November showed the most accelerated growth (Figures 4.1, 4.2, 4.3, 4.4, 4.5), because they were experiencing both N stress and quickened development from warming. Under lower N environments and later planting dates, the time between tillering and anthesis was reduced (Table 4.1, 4.2, and 4.6). Truman developed later than Pembroke across N

environments and planting dates, in terms of phyllochron, heading date and anthesis date (Table 4.1 and 4.6).

Effects of N Environment and Planting Date Measured Traits

Later planting dates resulted in plants being exposed to higher temperatures during the period between anthesis and harvest. Post-anthesis average maximum, minimum, and average temperature for the September, October, and November planting are as follows in °C: 25.4, 16.2, 20.8; 25.9, 16.3, 20.9; 26.4, 18.6, 21.5. Traits including yield, grain N content, and biomass were significantly related to temperature ($p<0.01$). traits including vegetative N content at anthesis and maturity, total plant N, PANU, NUpE, and NUE were also strongly correlated to temperature after anthesis ($p<0.01$) (Table 4.7). Higher temperatures resulted in decreased yield and grain N content, along with many of the N traits, including NUpE and overall NUE. Nitrogen utilization efficiency reduced under increased temperature, but the relationship was not significant, likely because there was less variation among the genotypes for NUtE than there was in the other N traits. Temperature did not have a positive effect on any of the traits measured (Table 4.2 and Table 4.7). In other wheat planting date studies as described below, similar effects have been seen in yield and yield components under later planting dates that subjected the plants to higher temperatures. In a particular planting window yield is optimized for a growing region. Early or late planting dates outside the optimum planting window can result in yield decreases due to unfavorable weather conditions, as in the later October and November plantings in the current study. In a similar planting date study, mean daily temperature between anthesis and harvest maturity for the 1st, 2nd, and 3rd planting date was 25, 28, and 31°C, respectively. Researchers found that as temperatures increased for each planting, yield declined. However, the relationship between temperature yield and yield components was not documented (Ouabbou and Paulsen, 2000). In another study, mean daily air temperatures during anthesis and grain filling were compared to yield components. There was shown to be a strong negative relationship between yield, kernel weight, and kernels per m^2 and temperature across planting dates (Ortiz-Monasterio *et al.*, 1994). A planting date study conducted by Ottman *et al.* (2012), compared average temperatures throughout the entire growing

season (pre-anthesis, post-anthesis, and all season temperature averages) to yield traits. Similar to our study, yield had a strong negative correlation to temperature, -0.94, though stronger than the relationship observed in our study, -0.74. All these studies are similar to the present study in that each one found higher temperatures under later planting dates caused yields to decrease and there was a negative relationship between temperature and yield and yield components. However, the effects of yield and other agronomic traits in response to increased temperature under various N rates was not investigated nor was N traits and the role these traits play in plant performance under elevated temperatures and multiple N environments.

In the present study, there was a significant effect between N environment and between planting date on each trait measured except for grain N (Table 4.2). The combined effect of both N environment and planting date was only significantly different for vegetative biomass at maturity, yield, grain N content, vegetative N at maturity, total plant N, NUpE and NUE. Genotypes (G) differed significantly for each trait measured (Table 4.2). There was a G*N environment interaction for traits NUpE and NUE. There was a G*planting date interaction for biomass traits, vegetative N traits, yield, grain N content, total plant N, NUpE, and NUE. The G*N environment*planting date interaction was significant ($P<0.05$) for yield, N grain content, total plant N, NUpE, and NUE (Table 4.1). The September planting date was subjected to lowest temperatures during the period from anthesis to harvest maturity compared to the other two planting dates across N environments. The November planting was subjected to the highest temperatures during this period (4.8, 4.9, and 4.10). Lower N regimes resulted in lower yield, biomass, grain N content and total plant N than higher N regimes as expected as did later planting dates. Plants in the later planting, low N environments; developed the earliest, had the lowest yields and grain N content, along with NUtE and NUE (Table 4.2. 4.3, 4.4, 4.5, 4.8, 4.9, and 4.10). This may be related to developmental differences: increased development may have reduced the length of time allowed for N remobilization to the grain, causing more N to be left in the biomass, thus causing a reduction in yield and grain N content (Table 4.2). NUpE was also lowest in the late planting date treatment (Table 4.2). Higher temperatures could have accelerated the time between tillering and anthesis, the time wheat plants take up N from the soil and store N in vegetative tissue. N

uptake largely stops once anthesis occurs and the N the plant has stored in vegetative biomass is remobilized to the grain. Therefore, plants may have had less time for N uptake, resulting in a lower NUpE and total plant. Shortened N uptake period may have also played a role in causing yield and grain N content to decrease.

Across N environments and planting dates NUpE had strong significant positive correlations to vegetative biomass at maturity, harvest index, yield, vegetative N content at maturity, N grain content, and total plant N across N environments and planting dates ($p<0.01$). Nitrogen use efficiency also had strong significant positive correlations to these traits as well. Across N environments and planting dates NUpE contributed significantly more to overall NUE than NUtE (Tables 4.11, 4.12, 4.13, 4.14, and 4.15). Correlations for these traits became stronger with later planting dates, indicating relationships under stress environments are more easily recognized. Under the early low to moderate N environments NUtE had a negative or barely positive correlation to yield, N grain content, and NUE. In the high N environments across planting dates, NUtE had a positive correlation to yield, grain N content, biomass at maturity, total plant N, and NUE; in the late planting date these correlations were significant ($p<0.01$) (Tables 4.11, 4.12, 4.13, 4.14, and 4.15). In the late planting date, grain filling was likely shortened thus reducing remobilization of N to the grain. Under late planting, genotypes with greater remobilization and NUtE would most likely be more advantageous in a warmer environment and have more of an impact on yield than in cooler environments, which are less stressed and would have a much longer grain filling period. Several studies have investigated the effects of warmer temperatures on N uptake and utilization in wheat (Jonassona *et al.* 2004; An *et al.* 2005; Yang *et al.* 2011). For instance, a night-time warming field experiment using reflective curtains showed that N accumulation in winter wheat during anthesis was 17-43% higher in the warmed than the unwarmed treatment. However, N utilization efficiency was decreased in the warmed treatment causing reduced N allocation towards yield during grain filling, resulting in a 6-25% yield decrease (Zhang *et al.* 2013). Giuliani *et al.* (2011) found that total plant N at anthesis and maturity, along with grain N has been shown to decrease under dryer conditions due to a lower NUpE and subsequent NUtE, especially at higher N levels. Overall, NUpE was more highly correlated to yield traits, N traits, and NUE than NUtE. Thus, focus on

enhancing NUpE may be beneficial in developing NUE winter wheat genotypes adapted to warmer environments.

Post-Anthesis N Uptake

As noted previously, N uptake occurs primarily prior to and during anthesis. However, some N may be taken up after anthesis (Gaju *et al.*, 2014). Post-anthesis N uptake (PANU) was significantly different between N environments and planting dates. PANU was significant at the $p<0.10$ at the genotype level (p value was 0.061; Table 4.2). Similarly, G*N environment interaction for PANU was significant at $P< 0.10$. However, there was no G*planting date interaction. There was a significant G*N environment*planting date interaction for PANU ($P<0.05$; Table 4.2). PANU was affected most under the high N environment across planting dates. In the high N environment, PANU was significantly positively correlated to yield ($p<0.05$), grain N content ($p<0.05$), NUpE ($p<0.05$), and total plant N ($p<0.05$) in the September and October planting dates (Tables 4.11-4.13). The moderate N environment had significant PANU only for the October planting date. Within the October planting moderate N environment, PANU was significantly correlated to yield ($p<0.05$), N grain content ($p<0.01$), total plant N content ($p<0.01$), and NUpE ($p<.01$) (Table 4.13). In the low N environment and November planting date, PANU for plants was minimal since development was quickened under these conditions and there was limited time available for N uptake after anthesis (Table 4.2). Also, under the low and moderate N environments there was less N supply, thus resulting in less opportunity for PANU than under the high N environment. Likely, the plants were able to uptake most all the N in the lower N environments prior to anthesis.

Correlation coefficients for traits associated with PANU ranged from 0.43-0.52 (Tables 4.11-4.15). These correlations, while significant, were not very strong; indicating PANU probably had a minor effect on genotypic performance for the high N environments and moderate N environments. However, other research has found that PANU contributes to plant performance. For instance, a study conducted using 16 wheat cultivars grown under varying N environments and multiple locations found that unfrt high N, PANU contributes 10.7% to grain N concetration and 14.4% in low N (Gaju *et*

al., 2014). Therefore, further investigation is needed to identify the influence of PANU using a larger number genotypes grown in research plots or head rows.

Root Biomass

Root biomass was measured for the genotypes Pembroke and Truman. These genotypes were expected differ in root biomass since both differ in height and development. However, the ANOVA showed no significant difference between genotypes across N environments and planting dates. Root biomass was significantly different between N treatments and planting dates ($p<0.01$). There was genotype*N environment ($p<0.05$) but no genotype*planting date interaction effect for root biomass. There was no genotype*N environment*planting date interaction (Table 4.16).

There was a significant positive correlation between N environment and root biomass ($p<0.01$) (Table 4.17), but there was no significant relationship with planting date. The results indicate that root biomass was affected more by N environment than planting date and temperature. There was a significant positive relationship between heading date and anthesis date for root biomass ($p<0.01$), plants with earlier heading dates and anthesis dates having larger root biomass. Root biomass was also positively correlated to vegetative biomass at maturity and anthesis, along with N concentration in the biomass at anthesis and maturity ($p<0.05$). Plants with larger root biomass are likely able to take up more nutrients, thus increasing vegetative biomass and vegetative N traits. Percent grain N and grain N content were also were shown to increase with increased biomass ($p<0.01$ and $p<0.05$, respectively). PANU also had a positive relationship to increased root biomass ($p<0.05$). Nitrogen uptake efficiency and NUE were not significantly correlated to root biomass. There are most likely other stronger factors that affect these traits such as N supply or temperature. Nitrogen utilization efficiency had a significant negative relationship with root biomass. Possibly more resources going towards root growth rather than aboveground plant growth reduces N remobilization to the grain. However, even though there were significant relationships between measured traits and root biomass, none of the correlations were very strong ranging from an R^2 of 0.27 to 0.39 (Table 4.17). Therefore, root biomass did not have a large effect on overall plant performance.

Plant N Availability from Resin Bag Data

Resin bags filled with mixed bed resin were used to determine plant N availability under each N environment planted on 27 September, 25 October, and 29 November 2012. Resin bags were sampled every two weeks. Nitrogen fertilizer was applied in a split application on 14 March and 4 April 2013 in the 101 and 168 kg N ha^{-1} environments. Results from the resin bag data are shown in figures 4.1, 4.2, and 4.3. Increases in N availability from N fertilizer application can be seen in figures 4.2 and 4.3. Under low N, the November planting date had more N available between 7 and 28 April, peaking on 18 April 2013 than the September and October planting dates, suggesting the plants in the late planting may have had lower N uptake due to accelerated development, causing more plant available N to be left behind in the soil and absorbed by the resin bags (Figure 4.1 and Table 4.18). In the 101 kg N ha^{-1} environment and the 168 kg N ha^{-1} more N was available under the September planting than the October and November planting. In the earlier planting dates, N uptake was higher as indicated by less N in resin bags after N application 14 March and 4 April 2013 under the moderate and high N environments (Figure 4.2 and 4.3, Table 4.2). Nitrogen availability measured by the resin bags was significantly different between N environments and sampling date ($p<0.0001$), but not between planting dates (Table 4.18). There were significant planting date*sampling date ($p<0.05$) and N environment*sampling date interaction ($p<0.0001$). There was no significant planting date*N environment*sampling date interactions (Table 4.18). Increases or decreases in N availability can also be caused by other processes such as mineralization, leaching, and denitrification, etc. that were not measured. As a result, not all trends observed in the resin bag data across N environments and planting dates can be described.

Genotypic Performance under Multiple N Environments and Warming

As N rate increased, significance at the genotypic level for both agronomic and N traits decreased (Tables 4.8, 4.9, and 4.10). This means that selection for yield, grain N content, and NUE traits may be more appropriate under N limiting environments rather than higher N environments. The October planting date showed the least genotypic

variation for all traits compared to the September and November planting dates (Tables 4.3, 4.4, and 4.5). The September planting date and November planting date showed similar genotypic variation across traits (Tables 4.3 and 4.5). However, the September planting had slightly higher genotypic variation for traits such as yield and total plant N. There was significantly higher genotypic variation for biomass traits, such as biomass at anthesis and maturity, HI, and vegetative N content at anthesis and maturity, and also NUtE for the September planting date (Table 4.3). Genotypic variation for overall NUE and NUpE was the same for the November and September planting dates. The November planting had higher genotypic variation for traits related to grain protein, percent grain N and grain N content (Tables 4.3 and 4.5). Plants sown in November tended to have higher percent grain N than plants planted in September, while September sown plants had much higher yields. Consistently, yield and grain N content decreased with lower N regimes and later planting dates (Tables 4.2, 4.3, 4.4, 4.5, 4.8, 4.9, and 4.10).

Truman was the most stable across N environments and planting dates in terms of yield, grain N content, and NUE. Truman consistently had the highest NUpE across N environments and planting dates (Tables 4.19 and 4.20). This is likely related to the fact that Truman is a late developing, photoperiod sensitive variety. As a result, this genotype most likely had a longer amount of time to uptake N from the soil probably due to an extended vegetative growth period. NUpE and total plant N had a strong positive correlation to NUE, yield and, grain N content across N environments and planting dates, Pearson correlations values ranging from 0.76-0.99 (Tables 4.11, 4.12, 4.13, 4.14, and 4.15). Lower NUpE meant lower yields and grain N content. This trend was consistent across N environments and locations. Truman may be a good candidate in the future to incorporate into breeding programs developing N use efficient lines adapted to warmer environments.

Table 4.1. Average leaf appearance based from phyllochron data over accumulated growing degree days (GDD) for soft red winter wheat genotype Truman (T) and Pembroke (P) planted September (S), October (O), and November (N) grown under three N environments: 0 kg N ha^{-1} (L), 101 kg N ha^{-1} (M), 168 kg N ha^{-1} (H) from the hill plot study Lexington, KY 2013. Level of significance from ANOVA shown below. N environment (E), planting date (PD), rep (R), genotype (G). Mean ($\bar{X}$) and standard error (SE) shown to the right.

	Genotype	T			P				
	GDD	L	M	H	L	M	H	$\bar{X}$	SE
	1614	4	3.3	3	4	3.8	3	3.5	0.19
	1712	4.3	4.7	3.3	4.3	4.3	3.7	4.1	0.21
	1820	4.8	4.8	4.2	5	4.7	4.2	4.6	0.14
N	1879	5	5.2	4.3	5.2	5.2	4.8	4.95	0.15
	1946	5.2	5.3	4.8	5.7	5.8	5	5.3	0.16
	2007	5.5	6	5.7	6	6	5.6	5.8	0.09
	2079	5.5	6	5.7	6	6	5.6	5.8	0.09
	2149	6	6	5.7	6	6	5.8	5.9	0.05
	2530	5	4.5	3.8	4.5	4.5	4.3	4.4	0.16
	2628	5.3	4.7	5	5.2	4.5	4.8	4.9	0.12
	2736	6	5.7	5.3	5.7	5.8	5.3	5.6	0.11
O	2795	6	5.7	5.7	5.8	5.8	5.8	5.8	0.04
	2862	6	5.7	6	5.8	5.8	6	5.9	0.05
	2923	6	6	6	6	5.8	6	6.0	0.03
	2995	6	6	6	6	5.8	6	6.0	0.03
	3065	6	6	6	6	6	6	6	0.00
	3531	5.2	5	4.7	5.2	5.3	5.8	5.2	0.15
	3629	5.3	5.3	5	6	5.7	6	5.6	0.17
	3737	6	6	6	6	6	6	6	0.00
S	3796	6	6	6	6	6	6	6	0.00
	3863	6	6	6	6	6	6	6	0.00
	3924	6	6	6	6	6	6	6	0.00
	3996	6	6	6	6	6	6	6	0.00
	4066	6	6	6	6	6	6	6	0.00

E	PD	E*PD	R(E)	G	E*G	PD*G	E*PD*G
0.0003	<0.0001	0.011	0.12	0.19	0.62	0.03	0.07

Table 4.2. LSMEANS agronomic trait and N trait averages for each N environment (N Env) and planting date (PD) (September (S), October (O), November (N)) from the hill plot study Lexington, KY 0 (L), 101 (M), and 168 (H) kg ha^{-1} N treatments. Level of significance from ANOVA is shown for each trait. Rep (R), N environment (Env), planting date (PD), genotype (G). Vegetative biomass anthesis (g m^{-2}), vegetative biomass maturity (g m^{-2}), yield (g m^{-2}), % grain N, grain N content (g m^{-2}), % N anthesis, % N maturity, post-anthesis N uptake (g m^{-2}), vegetative N at anthesis (g m^{-2}), vegetative N at maturity (g m^{-2}), total plant N (g m^{-2}), N uptake efficiency (NUpE), N utilization efficiency (NUtE)(g yield g^{-1} plant N), and N use efficiency (NUE).

N	Env	PD	HD	AD	Vba	Vma	HI	Y	Ng	Ngc	Na	Nm	PANU	Vna	Vnm	TN	NUpE	NUtE	NUE
		S	14	15	527.5	408.8	0.48	372.4	2.29	8.58	1.58	0.57	6.16	8.42	2.46	11.00	2.07	34.9	70.0
L		O	15	17	72.4	71.5	0.54	85.0	2.35	1.99	1.81	0.68	0.80	1.31	0.51	2.50	0.47	34.5	16.0
		N	20	22	33.2	18.7	0.57	25.1	2.37	0.58	2.19	0.77	0.58	0.74	0.14	0.73	0.14	33.8	4.7
		S	14	16	617.1	585.7	0.49	572.6	2.56	14.61	1.89	0.67	7.62	11.54	3.91	18.52	1.20	31.6	37.2
M		O	17	19	149.0	174.4	0.55	214.3	2.47	5.29	2.00	0.68	1.85	3.18	1.22	6.46	0.42	33.5	13.9
		N	21	23	117.5	72.2	0.53	82.9	2.53	2.09	2.36	0.91	1.89	2.64	0.68	2.78	0.18	30.2	5.4
		S	16	18	659.9	680.9	0.47	599.8	2.69	16.05	1.98	0.73	7.96	13.08	5.03	21.09	0.95	28.4	27.1
H		O	18	20	185.9	200.8	0.54	231.5	2.69	6.20	2.36	0.86	2.24	4.11	1.67	7.86	0.36	29.2	10.5
		N	22	24	129.2	104.7	0.53	117.0	2.71	3.13	2.51	1.05	2.25	3.20	1.02	4.21	0.19	27.1	5.3
		E	<.0001	<.0001	<.0001	<.0001	0.02	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
		PD	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.75	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.02	<.0001
		E*PD	0.10	0.02	0.78	<.0001	0.72	<.0001	0.72	<.0001	0.38	0.47	0.99	0.06	0.0003	<.0001	<.0001	0.60	<.0001
		R(E)	0.64	0.81	<.0001	<.0001	0.32	<.0001	0.004	0.006	0.39	0.53	<.0001	<.0001	0.003	0.001	0.0003	0.26	<.0001
		G	<.0001	<.0001	<.0001	0.004	0.001	<.0001	0.001	<.0001	0.003	0.002	0.06	0.001	0.0001	<.0001	<.0001	<.0001	<.0001
		G*E	0.59	0.45	0.67	0.85	0.55	0.472	0.12	0.15	0.38	0.49	0.06	0.16	0.31	0.53	0.002	0.36	0.006
		G*PD	0.01	0.54	0.0003	0.012	0.17	<.0001	0.60	0.001	0.001	0.73	0.17	0.005	0.013	0.002	0.0003	0.49	<.0001
		G*E*PD	0.02	0.002	0.28	0.18	0.18	0.026	0.25	0.01	0.52	0.88	0.03	0.14	0.16	0.03	0.001	0.95	0.001

Table 4.3. LSMEANS agronomic trait and N trait averages for each N environment (Env) under the September planting date from the hill plot study Lexington, KY 0 (L), 101 (M), and 168 (H) kg ha^{-1} N treatments. Level of significance from ANOVA is shown for each trait. Rep (R), N environment (Env), genotype (G). Vegetative biomass anthesis (g m^{-2}), vegetative biomass maturity (g m^{-2}), yield (g m^{-2}), % grain N, grain N content (g m^{-2}), % N anthesis, % N maturity, post-anthesis N uptake (g m^{-2}), vegetative N at anthesis (g m^{-2}), vegetative N at maturity (g m^{-2}), total plant N (g m^{-2}), N uptake efficiency (NUpE), N utilization efficiency (NUtE)(g yield g^{-1} plant N), and N use efficiency (NUE).

ENV	Vba	Vma	HI	Y	Ng	Ngc	Na	Nm	PANU	Vna	Vnm	TN	NUpE	NUtE	NUE
L	527.5	408.8	0.41	372.4	2.29	8.58	1.58	0.57	6.16	8.42	2.46	11.00	2.07	34.9	70.0
M	617.1	585.7	0.48	572.6	2.56	14.61	1.89	0.67	7.62	11.54	3.91	18.52	1.20	31.6	37.2
H	659.9	680.9	0.48	599.8	2.69	16.05	1.98	0.73	7.96	13.08	5.03	21.09	0.95	28.4	27.1
Env	0.0002	<.0001	0.0002	<.0001	0.005	<.0001	0.001	0.05	0.05	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
R(Env)	0.15	0.14	0.10	0.05	0.23	0.86	0.52	0.80	0.11	0.03	0.41	0.72	0.13	0.66	<.0001
G	0.0003	0.02	<.0001	<.0001	0.14	0.0004	0.21	0.07	0.12	0.002	0.01	0.001	0.001	0.004	<.0001
G*Env	0.65	0.32	0.20	0.06	0.33	0.03	0.23	0.40	0.04	0.10	0.24	0.12	0.01	0.71	0.001

Table 4.4. LSMEANS agronomic trait and N trait averages for each N environment (Env) under the October planting date from the hill plot study Lexington, KY 0 (L), 101 (M), and 168 (H) kg ha^{-1} N treatments. Level of significance from ANOVA is shown for each trait. Rep (R), N environment (Env), genotype (G). Vegetative biomass anthesis (g m^{-2}), vegetative biomass maturity (g m^{-2}), yield (g m^{-2}), % grain N, grain N content (g m^{-2}), % N anthesis, % N maturity, post-anthesis N uptake (g m^{-2}), vegetative N at anthesis (g m^{-2}), vegetative N at maturity (g m^{-2}), total plant N (g m^{-2}), N uptake efficiency (NUpE), N utilization efficiency (NUtE)(g yield g^{-1} plant N), and N use efficiency (NUE).

ENV	Vba	Vma	HI	Y	Ng	Ngc	Na	Nm	PANU	Vna	Vnm	TN	NUpE	NUtE	NUE
L	72.4	71.5	0.54	85.0	2.35	1.99	1.81	0.68	0.80	1.31	0.51	2.50	0.47	34.5	16.0
M	149.0	174.4	0.61	214.3	2.47	5.29	2.00	0.68	1.85	3.18	1.22	6.46	0.42	33.5	13.9
H	185.9	200.8	0.56	231.5	2.69	6.20	2.36	0.86	2.24	4.11	1.67	7.86	0.36	29.2	10.5
Env	<.0001	<.0001	0.18	<.0001	<.0001	<.0001	0.0003	0.001	0.005	<.0001	<.0001	<.0001	0.02	<.0001	0.0002
R(Env)	<.0001	0.001	0.40	0.001	0.15	0.001	0.83	0.64	0.001	<.0001	0.004	0.002	0.0001	0.28	<.0001
G	0.03	0.11	0.51	0.04	0.57	0.03	0.10	0.01	0.35	0.18	0.06	0.06	0.002	0.06	0.002
G*Env	0.71	0.49	0.72	0.37	0.69	0.44	0.68	0.16	0.26	0.72	0.21	0.45	0.14	0.39	0.20

Table 4.5. LSMEANS agronomic trait and N trait averages for each N environment (Env) under the November planting date from the hill plot study Lexington, KY 0 (L), 101 (M), and 168 (H) kg ha $^{-1}$ N treatments. Level of significance from ANOVA is shown for each trait. Rep (R), N environment (Env), genotype (G). Vegetative biomass anthesis (g m^{-2}), vegetative biomass maturity (g m^{-2}), yield (g m^{-2}), % grain N, grain N content (g m^{-2}), % N anthesis, % N maturity, post-anthesis N uptake (g m^{-2}), vegetative N at anthesis (g m^{-2}), vegetative N at maturity (g m^{-2}), total plant N (g m^{-2}), N uptake efficiency (NUpE), N utilization efficiency (NUtE) (g yield g^{-1} plant N), and N use efficiency (NUE).

ENV	Vba	Vma	HI	Y	Ng	Ngc	Na	Nm	PANU	Vna	Vnm	TN	NUpE	NUtE	NUE
L	33.2	18.7	0.41	25.1	2.37	0.58	2.19	0.77	0.58	0.74	0.14	0.73	0.14	33.8	4.7
M	117.5	72.5	0.44	82.9	2.53	2.09	2.36	0.91	1.89	2.64	0.68	2.78	0.18	30.2	5.4
H	129.2	104.7	0.43	117.0	2.71	3.13	2.51	1.05	2.25	3.20	1.02	4.21	0.19	27.1	5.3
Env	<.0001	<.0001	0.82	<.0001	<.0001	<.0001	0.03	0.01	0.01	<.0001	<.0001	<.0001	0.09	0.0007	0.58
R(Env)	0.0001	<.0001	0.44	<.0001	0.09	<.0001	0.62	0.41	0.05	0.0006	0.0007	<.0001	0.0002	0.29	<.0001
G	0.09	0.01	0.06	0.0002	0.01	0.0002	0.001	0.66	0.46	0.26	0.17	0.003	0.001	0.15	0.0001
G*Env	0.09	0.71	0.19	0.41	0.06	0.39	0.68	0.98	0.49	0.31	0.83	0.58	0.90	0.78	0.95

Table 4.6. Heading date (HD) and anthesis date (AD) LSMEANS for soft red winter lines Pembroke and Truman planted September (S), October (O), and November (N) under 0 kg N ha^{-1} (L), 101 kg N ha^{-1} (M), 168 kg N ha^{-1} (H) from the hill plot study Lexington, KY 2013.

Genotype	PD	L		M		H	
		HD	AD	HD	AD	HD	AD
Pembroke	S	13	14	14	15	15	16
	O	14	15	15	18	18	20
	N	19	21	19	22	20	22
Truman	S	14	16	15	17	17	20
	O	16	19	17	20	18	20
	N	21	23	22	24	23	25
	$\bar{x}$	16	18	17	19	18	20
	SE	1.3	1.5	1.3	1.4	1.2	1.3

Table 4.7. Pearson correlation coefficients of measured traits and average maximum temperature (Tmax), average minimum temperature (Tmin), average temperature (Tavg) for the period between anthesis and harvest maturity for winter wheat planted September 27 (S), October 25 (O), and November (N) 29, 2012. Average anthesis date for each planting date is as follows: May 17, May 19, May 22. Harvest maturity of each planting is as follows: June 25, July 2, July 12, respectively.

	Vba	Vbm	Y	Vna	Vnm	Ngc	TN	PANU	NUpE	NUtE	NUE
Tmax	-0.74**	-0.75**	-0.75**	-0.67**	-0.57**	-0.72**	-0.73**	-0.66**	-0.63**	-0.05	-0.67**
Tmin	-0.56**	-0.55**	-0.58**	-0.50**	-0.41**	-0.56**	-0.53**	-0.49**	-0.48**	-0.12	-0.51**
Tavg	-0.56**	-0.55**	-0.58**	-0.50**	-0.41**	-0.56**	-0.53**	-0.49**	-0.48**	-0.12	-0.51**

Table 4.8. LSMEANS agronomic trait and N trait averages for each planting date (PD) (September (S), October (O), November (N)) from the hill plot study Lexington, KY 0 kg ha^{-1} N treatment. Level of significance from ANOVA is shown for each trait. Rep (R), planting date (PD), genotype (G). Vegetative biomass anthesis (g m^{-2}), vegetative biomass maturity (g m^{-2}), yield (g m^{-2}), % grain N, grain N content (g m^{-2}), % N anthesis, % N maturity, post-anthesis N uptake (g m^{-2}), vegetative N at anthesis (g m^{-2}), vegetative N at maturity (g m^{-2}), total plant N (g m^{-2}), N uptake efficiency (NUpE), N utilization efficiency (NUtE) (g yield g^{-1} plant N), and N use efficiency (NUE).

PLD	Vba	Vma	HI	Y	Ng	Ngc	Na	Nm	PANU	Vna	Vnm	TN	NUpE	NUtE	NUE
S	527.5	408.8	0.41	372.4	2.29	8.58	1.58	0.57	6.16	8.42	2.46	11.00	2.07	34.9	70.0
O	72.4	71.5	0.54	85.0	2.35	1.99	1.81	0.68	0.80	1.31	0.51	2.50	0.47	34.5	16.0
N	33.2	18.7	0.41	25.1	2.37	0.58	2.19	0.77	0.58	0.74	0.14	0.73	0.14	33.8	4.7
PLD	<.0001	<.0001	0.003	<.0001	0.64	<.0001	<.0001	0.01	<.0001	<.0001	<.0001	<.0001	<.0001	0.76	<.0001
R(PLD)	0.09	0.01	0.81	<.0001	0.18	0.08	0.84	0.59	0.74	0.22	0.19	0.03	0.03	0.28	<.0001
G	0.01	0.08	0.25	<.0001	0.04	0.0002	0.33	0.39	0.23	0.12	0.03	0.0002	0.0002	0.04	<.0001
G*PLD	0.05	0.41	0.95	<.0001	0.42	0.004	0.22	0.38	0.38	0.24	0.15	0.004	0.004	0.67	<.0001

Table 4.9. LSMEANS agronomic trait and N trait averages for each planting date (PD) (September (S), October (O), November (N)) from the hill plot study Lexington, KY 101 kg ha^{-1} N treatment. Level of significance from ANOVA is shown for each trait. Rep (R), planting date (PD), genotype (G). Vegetative biomass anthesis (g m^{-2}), vegetative biomass maturity (g m^{-2}), yield (g m^{-2}), % grain N, grain N content (g m^{-2}), % N anthesis, % N maturity, post-anthesis N uptake (g m^{-2}), vegetative N at anthesis (g m^{-2}), vegetative N at maturity (g m^{-2}), total plant N (g m^{-2}), N uptake efficiency (NUpE), N utilization efficiency (NUtE) (g yield g^{-1} plant N), and N use efficiency (NUE).

PLD	Vba	Vma	HI	Y	Ng	Ngc	Na	Nm	PANU	Vna	Vnm	TN	NUpE	NUtE	NUE
S	617.1	585.7	0.48	572.6	2.56	14.61	1.89	0.67	7.62	11.54	3.91	18.52	1.20	31.6	37.2
O	149.0	174.4	0.61	214.3	2.47	5.29	2.00	0.68	1.85	3.18	1.22	6.46	0.42	33.5	13.9
N	117.5	72.5	0.44	82.9	2.53	2.09	2.36	0.91	1.89	2.64	0.68	2.78	0.18	30.2	5.4
PLD	<.0001	<.0001	<.0001	<.0001	0.5818	<.0001	0.0007	0.0058	<.0001	<.0001	<.0001	<.0001	<.0001	0.0583	<.0001
R(PLD)	0.01	0.01	0.38	0.002	0.13	0.12	0.53	0.74	0.06	0.001	0.09	0.11	0.11	0.72	0.002
G	0.03	0.13	0.32	0.01	0.03	0.01	0.04	0.40	0.11	0.005	0.17	0.02	0.02	0.02	0.01
G*PLD	0.03	0.01	0.01	0.001	0.36	0.003	0.11	0.98	0.10	0.01	0.65	0.01	0.01	0.84	0.001

Table 4.10. LSMEANS agronomic trait and N trait averages for each planting date (PD) (September (S), October (O), November (N)) from the hill plot study Lexington, KY 168 kg N ha^{-1} environment. Level of significance from ANOVA is shown for each trait. Rep (R), planting date (PD), genotype (G). Vegetative biomass anthesis (g m^{-2}), vegetative biomass maturity (g m^{-2}), yield (g m^{-2}), % grain N, grain N content (g m^{-2}), % N anthesis, % N maturity, post-anthesis N uptake (g m^{-2}), vegetative N at anthesis (g m^{-2}), vegetative N at maturity (g m^{-2}), total plant N (g m^{-2}), N uptake efficiency (NUpE), N utilization efficiency (NUtE) (g yield g^{-1} plant N), and N use efficiency (NUE).

PLD	Vba	Vma	HI	Y	Ng	Ngc	Na	Nm	PANU	Vna	Vnm	TN	NUpE	NUtE	NUE
S	659.9	680.9	0.48	599.8	2.69	16.05	1.98	0.73	7.96	13.08	5.03	21.09	0.95	28.4	27.1
O	185.9	200.8	0.56	231.5	2.69	6.20	2.36	0.86	2.24	4.11	1.67	7.86	0.36	29.2	10.5
N	129.2	104.7	0.43	117.0	2.71	3.13	2.51	1.05	2.25	3.20	1.02	4.21	0.19	27.1	5.3
PLD	<.0001	<.0001	0.001	<.0001	0.93	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.05	<.0001
R(PLD)	<.0001	0.06	0.08	0.03	0.36	0.08	0.67	0.28	0.003	0.001	0.16	0.06	0.06	0.15	0.03
G	0.08	0.33	0.01	0.01	0.28	0.01	0.21	0.004	0.12	0.33	0.01	0.17	0.17	0.01	0.01
G*PLD	0.32	0.33	0.09	0.05	0.51	0.16	0.16	0.67	0.24	0.52	0.01	0.36	0.36	0.46	0.05

Table 4.11. Pearson correlation coefficients for agronomic and N traits measured from the 8 soft red winter wheat lines grown under 0 kg N ha^{-1} and 101 kg N ha^{-1} environments hill plot study 2013 September planting. 0 kg N ha^{-1} environment correlations depicted above the diagonal and 101 kg N ha^{-1} environment below. Vegetative biomass at anthesis (Vba), vegetative biomass at maturity (Vbm), yield (Y), harvest index (HI), % grain N (Ng), % N anthesis in vegetative tissue (Na), % N maturity vegetative tissue (Nm), grain N content (Ngc), vegetative N anthesis content (Vna), vegetative N maturity content (Vnm), total plant N content (TN), post-anthesis N uptake (PANU), nitrogen use efficiency (NUE), nitrogen utilization efficiency (NUtE), nitrogen uptake efficiency (NUpE).

	Vbm	Vba	HI	Y	Na	Nm	Ng	Vna	Vnm	Ngc	TN	PANU	NUpE	NUtE	NUE
Vbm	.	0.26	0.53**	0.74**	0.05	0.25	0.19	0.19	0.63**	0.65**	0.73**	-0.20	0.73**	-0.29	0.74**
Vba	-0.14	.	-0.48*	0.06	0.18	0.40	0.29	0.75*	0.48*	0.22	0.32	0.70**	0.32	-0.35	0.06
HI	0.65**	-0.79**	.	0.84**	-0.03	-0.10	-0.08	-0.32	0.15	0.57**	0.56**	-0.50*	0.56**	0.19	0.84**
Y	0.91**	-0.21	0.75**	.	0.09	0.08	0.12	0.09	0.41	0.81**	0.80**	-0.15	0.80**	0.02	1.00**
Na	0.24	-0.17	0.20	0.14	.	0.70**	0.11	0.78**	0.64**	0.14	0.33	0.68**	0.33	-0.38	0.09
Nm	0.06	0.10	-0.04	0.07	0.14	.	0.13	0.72**	0.89**	0.15	0.41	0.38	0.41	-0.59**	0.08
Ng	0.09	-0.11	0.06	-0.05	0.35	-0.15	.	0.30	0.23	0.68**	0.63**	0.24	0.63**	-0.80**	0.12
Vna	0.11	0.62**	-0.43**	-0.02	0.66**	0.17	0.16	.	0.73**	0.26	0.44*	0.88**	0.44*	-0.50*	0.09
Vnm	0.61**	0.00	0.33	0.57**	0.27	0.80**	-0.06	0.22	.	0.44	0.68**	0.32	0.68**	-0.61**	0.41
Ngc	0.73**	-0.20	0.57**	0.71**	0.36	-0.01	0.66**	0.13	0.42*	.	0.96**	0.04	0.96**	-0.45*	0.81**
TN	0.80**	-0.16	0.58**	0.76**	0.38	0.24	0.53**	0.18	0.66**	0.96**	.	0.14	1.00**	-0.57**	0.80**
PANU	-0.17	0.62**	-0.58**	-0.28	0.53**	-0.20	0.18	0.89**	-0.24	-0.06	-0.12	.	0.14	-0.27	-0.15
NUpE	0.80**	-0.16	0.58**	0.76**	0.38	0.24	0.53**	0.18	0.66**	0.96**	1.00**	-0.12	.	-0.57**	0.80**
NUtE	-0.09	-0.03	0.05	0.06	-0.38	-0.40	-0.80**	-0.29	-0.38	-0.55**	-0.57**	-0.11	-0.57**	.	0.02
NUE	0.91**	-0.21	0.75**	1.00**	0.14	0.07	-0.05	-0.02	0.57**	0.71**	0.76**	-0.28	0.76**	0.06	.

*p<0.05, **p<0.01

Table 4.12. Pearson correlation coefficients for agronomic and N traits measured from the 8 soft red winter wheat lines planted September and October hill plot study 2013 168 kg N ha^{-1} environment. September planting correlations depicted above the diagonal and October planting below. Vegetative biomass at anthesis (Vba), vegetative biomass at maturity (Vbm), yield (Y), harvest index (HI), % grain N (Ng), % N anthesis in vegetative tissue (Na), % N maturity vegetative tissue (Nm), grain N content (Ngc), vegetative N anthesis content (Vna), vegetative N maturity content (Vnm), total plant N content (TN), post-anthesis N uptake (PANU), nitrogen use efficiency (NUE), nitrogen utilization efficiency (NUtE), nitrogen uptake efficiency (NUpE).

	Vbm	Vba	HI	Y	Na	Nm	Ng	Vna	Vnm	Ngc	TN	PANU	NUpE	NUtE	NUE
Vbm	.	0.40	0.00	0.38	0.44**	0.22	-0.03	0.56**	0.72**	0.38	0.67**	0.17	0.67**	-0.39	0.38
Vba	0.48*	.	-0.54**	0.13	0.06	0.37	0.24	0.79**	0.44*	0.22	0.41	0.59**	0.41	-0.39	0.13
HI	0.29	-0.63**	.	0.75**	0.34	-0.51*	-0.33	-0.19	-0.32	0.66**	0.43*	-0.01	0.43*	0.76**	0.75**
Y	0.97**	0.48*	0.32	.	0.49*	-0.27	-0.22	0.41	0.01	0.94**	0.85**	0.43*	0.85**	0.55**	1.00**
Na	0.35	-0.05	0.51*	0.40	.	-0.09	-0.12	0.66**	0.20	0.46*	0.51*	0.59**	0.51*	0.12	0.49*
Nm	-0.35	-0.44*	0.18	-0.38	0.15	.	0.03	0.19	0.83**	-0.33	0.04	-0.30	0.04	-0.71	-0.27
Ng	-0.18	-0.16	0.04	-0.25	-0.19	0.25	.	0.11	-0.02	0.11	0.09	0.13	0.09	-0.53*	-0.22
Vna	0.63**	0.97**	-0.51*	0.65**	0.20	-0.38	-0.22	.	0.42*	0.45*	0.61**	0.83**	0.61**	-0.19	0.41
Vnm	0.78**	0.18	0.43*	0.74**	0.42	0.25	-0.16	0.33	.	-0.01	0.42	-0.15	0.42	-0.71**	0.01
Ngc	0.97**	0.48*	0.33	0.99**	0.37	-0.37	-0.13	0.65**	0.73**	.	0.90**	0.52*	0.90**	0.38	0.94**
TN	0.97**	0.44*	0.36	0.98**	0.40	-0.26	-0.14	0.62**	0.82**	0.99**	.	0.43*	1.00**	0.04	0.85**
PANU	0.46*	0.95**	-0.62**	0.49*	0.09	-0.48*	-0.19	0.96**	0.06	0.49*	0.43*	.	0.43*	0.21	0.43*
NUpE	0.97**	0.44*	0.36	0.98**	0.40	-0.26	-0.14	0.62**	0.82**	0.99**	1.00**	0.43*	.	0.04	0.85**
NUtE	0.25	0.33	-0.07	0.37	0.05	-0.80**	-0.72**	0.33	-0.16	0.30	0.22	0.40	0.22	.	0.55**
NUE	0.97**	0.48*	0.32	1.00**	0.40	-0.38	-0.25	0.65**	0.74**	0.99**	0.98**	0.49*	0.98**	0.37	.

*p<0.05, **p<0.01

Table 4.13. Pearson correlation coefficients for agronomic and N traits measured from the 8 soft red winter wheat lines grown under 0 kg N ha^{-1} and 101 kg N ha^{-1} environments hill plot study 2013 October planting. 0 kg N ha^{-1} environment correlations depicted above the diagonal and 101 kg N ha^{-1} environment below. Vegetative biomass at anthesis (Vba), vegetative biomass at maturity (Vbm), yield (Y), harvest index (HI), % grain N (Ng), % N anthesis in vegetative tissue (Na), % N maturity vegetative tissue (Nm), grain N content (Ngc), vegetative N anthesis content (Vna), vegetative N maturity content (Vnm), total plant N content (TN), post-anthesis N uptake (PANU), nitrogen use efficiency (NUE), nitrogen utilization efficiency (NUtE), nitrogen uptake efficiency (NUpE).

	Vbm	Vba	HI	Y	Na	Nm	Ng	Vna	Vnm	Ngc	TN	PANU	NUpE	NUtE	NUE
Vbm	.	0.25	0.52**	0.92**	0.21	0.39	-0.09	0.31	0.91**	0.89**	0.94**	-0.14	0.94**	-0.42	0.92**
Vba	0.70**	.	-0.58**	0.17	0.04	0.39	0.05	0.85**	0.33	0.19	0.25	0.78**	0.25	-0.43*	0.17
HI	-0.13	-0.70**	.	0.64**	0.21	-0.13	-0.05	-0.37	0.35	0.62**	0.57**	-0.60**	0.57**	0.18	0.64**
Y	0.92**	0.68**	-0.03	.	0.23	0.29	-0.06	0.27	0.81**	0.98**	0.98**	-0.13	0.98**	-0.23	1.00**
Na	0.25	0.28	-0.08	0.25	.	0.24	-0.10	0.53**	0.28	0.23	0.25	0.44*	0.25	-0.10	0.23
Nm	0.19	0.31	-0.38	0.12	0.36	.	-0.13	0.53**	0.72**	0.27	0.42*	0.21	0.42*	-0.74**	0.29
Ng	0.06	0.31	-0.41*	0.07	0.21	0.40	.	0.001	-0.14	0.12	0.04	0.07	0.04	-0.44*	-0.06
Vna	0.68**	0.94**	-0.62**	0.66**	0.56**	0.34	0.30	.	0.45*	0.28	0.35	0.88**	0.35	-0.44*	0.27
Vnm	0.82**	0.70**	-0.27	0.72**	0.34	0.68**	0.16	0.67**	.	0.77**	0.89**	-0.03	0.88**	-0.60**	0.81**
Ngc	0.91**	0.73**	-0.10	0.99**	0.28	0.18	0.21	0.71**	0.76**	.	0.98**	-0.10	0.98**	-0.31	0.98**
TN	0.94**	0.69**	-0.06	0.97**	0.31	0.32	0.12	0.66**	0.86**	0.98**	.	-0.08	1.00**	-0.42*	0.98**
PANU	0.46*	0.85**	-0.62**	0.41*	0.61**	0.16	0.14	0.96**	0.42*	0.44**	0.45*	.	-0.08	-0.16	-0.13
NUpE	0.94**	0.69**	-0.06	0.97**	0.31	0.32	0.12	0.66**	0.86**	0.98**	1.00**	0.45**	.	-0.42*	0.98**
NUtE	-0.22	-0.39	0.51**	-0.09	-0.31	-0.83**	-0.80**	-0.37	-0.59**	-0.20	-0.32	-0.23	-0.32	.	-0.23
NUE	0.92**	0.68**	-0.03	1.00**	0.25	0.12	0.07	0.66**	0.72**	0.99**	0.97**	0.41	0.97**	-0.09	.

*p<0.05, **p<0.01

Table 4.14. Pearson correlation coefficients for agronomic and N traits measured from the 8 soft red winter wheat lines grown under 0 kg N ha^{-1} and 101 kg N ha^{-1} environments hill plot study 2013 November planting. 0 kg N ha^{-1} environment correlations depicted above the diagonal and 101 kg N ha^{-1} environment below. Vegetative biomass at anthesis (Vba), vegetative biomass at maturity (Vbm), yield (Y), harvest index (HI), % grain N (Ng), % N anthesis in vegetative tissue (Na), % N maturity vegetative tissue (Nm), grain N content (Ngc), vegetative N anthesis content (Vna), vegetative N maturity content (Vnm), total plant N content (TN), post-anthesis N uptake (PANU), nitrogen use efficiency (NUE), nitrogen utilization efficiency (NUtE), nitrogen uptake efficiency (NUpE).

	Vbm	Vba	HI	Y	Na	Nm	Ng	Vna	Vnm	Ngc	TN	PANU	NUpE	NUtE	NUE
Vbm	.	0.21	0.64**	0.86**	-0.25	-0.07	-0.31	0.14	0.89**	0.84**	0.92**	-0.11	0.92**	0.14	0.86**
Vba	0.39	.	-0.21	0.46*	-0.19	-0.39	-0.19	0.89**	0.03	0.43*	0.36	0.83**	0.36	0.42*	0.46*
HI	0.24	-0.42*	.	0.68**	-0.27	-0.18	-0.25	-0.28	0.55**	0.69**	0.70**	-0.42	0.70**	0.38	0.68**
Y	0.91**	0.52**	0.30	.	-0.31	-0.40	-0.33	0.35	0.61**	0.98**	0.97**	0.17	0.97**	0.47**	1.00**
Na	-0.14	-0.24	0.25	-0.28	.	0.04	-0.24	0.27	-0.26	-0.37	-0.34	0.34	-0.34	0.07	-0.31
Nm	0.14	-0.17	-0.11	-0.04	-0.004	.	-0.17	-0.36	0.34	-0.42*	-0.26	-0.44*	-0.26	-0.56**	-0.40
Ng	0.05	-0.22	-0.26	-0.10	-0.21	0.24	.	-0.28	-0.32	-0.19	-0.24	-0.18	-0.24	-0.57**	-0.33
Vna	0.38	0.97**	-0.40*	0.45*	-0.04	-0.18	-0.22	.	-0.05	0.31	0.25	0.96**	0.25	0.44*	0.35
Vnm	0.90**	0.23	0.07	0.75**	-0.14	0.50*	0.27	0.22	.	0.59**	0.73**	-0.33	0.73**	-0.08	0.61**
Ngc	0.93**	0.47*	0.31	0.99**	-0.28	0.01	0.02	0.41*	0.80**	.	0.98**	0.14	0.98**	0.41	0.98**
TN	0.96**	0.41*	0.14	0.95**	-0.27	0.18	0.17	0.36	0.91**	0.98**	.	0.04	1.00**	0.32	0.97**
PANU	0.15	0.93**	-0.58**	0.27	-0.01	-0.32	-0.29	0.97**	-0.04	0.21	0.13	.	0.04	0.43*	0.17
NUpE	0.96**	0.41	0.14	0.95**	-0.27	0.18	0.17	0.36	0.91**	0.98**	1.00**	0.13	.	0.32	0.97**
NUtE	-0.15	0.25	0.33	0.18	0.04	-0.80**	-0.59**	0.22	-0.43*	0.09	-0.09	0.34	-0.09	.	0.47*
NUE	0.91**	0.52**	0.30	1.00**	-0.28	-0.04	-0.10	0.45*	0.75*	0.99**	0.95**	0.27	0.95**	0.18	.

*p<0.05, **p<0.01

Table 4.15. Pearson correlation coefficients for agronomic and N traits measured from the 8 soft red winter wheat lines grown under 168 kg N ha^{-1} environments hill plot study 2013 November planting. Vegetative biomass at anthesis (Vba), vegetative biomass at maturity (Vbm), yield (Y), harvest index (HI), % grain N (Ng), % N anthesis in vegetative tissue (Na), % N maturity vegetative tissue (Nm), grain N content (Ngc), vegetative N anthesis content (Vna), vegetative N maturity content (Vnm), total plant N content (TN), post-anthesis N uptake (PANU), nitrogen use efficiency (NUE), nitrogen utilization efficiency (NUtE), nitrogen uptake efficiency (NUpE).

	Vbm	Vba	HI	Y	Na	Nm	Ng	Vna	Vnm	Ngc	TN	PANU	NUpE	NUtE	NUE
Vbm	.	0.73**	0.58**	0.93**	-0.49*	-0.41	-0.27	0.63**	0.83**	0.91**	0.95**	0.41	0.95**	0.51*	0.93**
Vba	.	.	0.16	0.64**	-0.29	-0.29	-0.29	0.96**	0.71**	0.63**	0.67**	0.85**	0.67**	0.23	0.64**
HI	.	.	.	0.75**	-0.34	-0.47*	-0.29	0.09	0.43*	0.76**	0.69**	-0.25	0.69**	0.64**	0.75**
Y	.	.	.	.	-0.46*	-0.48	-0.32	0.55**	0.75**	0.99**	0.99**	0.30	0.99**	0.63**	1.00**
Na	.	.	.	.	.	0.20	0.20	-0.03	-0.47*	-0.44*	-0.50*	0.16	-0.50*	-0.39	-0.46*
Nm	.	.	.	.	.	.	0.16	-0.27	0.07	-0.51*	-0.35	-0.28	-0.35	-0.82**	-0.48*
Ng	.	.	.	.	.	.	.	-0.22	-0.38	-0.26	-0.19	0.04	-0.19	-0.35	-0.32
Vna	.	.	.	.	.	.	.	.	0.62**	0.55**	0.57**	0.94**	0.57**	0.15	0.55**
Vnm	.	.	.	.	.	.	.	.	.	0.71**	0.83**	0.33	0.83**	0.18	0.75**
Ngc	.	.	.	.	.	.	.	.	.	.	0.98**	0.32	0.98**	0.64**	0.99**
TN	.	.	.	.	.	.	.	.	.	.	.	0.34	1.00**	0.55**	0.99**
PANU	.	.	.	.	.	.	.	.	.	.	.	.	0.34	0.11	0.30
NUpE	.	.	.	.	.	.	.	.	.	.	.	.	.	0.55**	0.99**
NUtE	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.63**
NUE	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

*p<0.05, **p<0.01

Table 4.16. LSMEANS root biomass (g m^{-2}) for genotypes Pembroke and Truman for each N environment (E) and planting date (PD) (September (S), October (O), November (N)) from the hill plot study Lexington, KY 0 (L), 101 (M), and 168 (H) kg N ha^{-1} treatments. Level of significance from ANOVA is shown for each trait. Rep (R), N environment (E), planting date (PD), genotype (G).

		Pembroke	Truman
E	PD	Root biomass	
L	S	4.61	3.48
	O	2.17	3.14
	N	0.26	0.71
M	S	9.26	3.17
	O	5.28	4.00
	N	3.90	2.81
H	S	5.52	6.26
	O	3.72	9.58
	N	3.00	4.25
	E		0.01
	PD		0.01
	E*PD		0.84
	R(E)		0.04
	G		0.96
	G*E		0.03
	G*PD		0.12
	G*E*PD		0.76

Table 4.17. Pearson correlation coefficients between root biomass (Rb), N environment (E), planting date (PD), and measured traits. Heading date (Hd), anthesis date (Ad), vegetative biomass at maturity (Vbm), vegetative biomass at anthesis (Vba), harvest index (HI), yield (Y), % N anthesis (Na), % N maturity (Nm), % grain N (Ng), total plant N (TN), post-anthesis nitrogen uptake (PANU), nitrogen uptake efficiency (NUpE), nitrogen utilization efficiency (NUtE), nitrogen use efficiency (NUE).

	E	PD	Hd	Ad	Vbm	Vba	HI	Y	Na	Nm	Ng	Vna	Vnm	Ngc	TN	PANU	NUpE	NUtE	NUE
Rb	0.36**	0.004	0.36**	0.39**	0.27*	0.26	-0.004	0.26	0.28*	0.10	0.39**	0.33*	0.29*	0.32*	0.33*	0.32*	-0.04	-0.37**	-0.08

Table 4.18. LSMEANS for resin bag data (ppm) for genotypes Pembroke and Truman for each N environment (E) and planting date (PD) (September (S), October (O), November (N)) from the hill plot study Lexington, KY 0 (L), 101 (M), and 168 (H) kg N ha^{-1} treatments. Level of significance from ANOVA is shown for each trait. Rep (R), N environment (E), planting date (PD), Sampling date (S).

S	E	PD	NH^{4+}	NO^{3-}	Total N
		S	1.6	4.7	6.2
	L	O	1.2	3.1	4.3
		N	1.5	4.3	5.8
		S	46.5	12.0	58.4
10-Mar	M	O	38.9	9.4	48.2
		N	16.5	11.1	27.6
		S	68.9	8.2	77.1
	H	O	60.2	6.8	67.0
		N	43.1	9.2	52.3
		S	2.2	3.3	5.5
	L	O	9.3	1.7	11.0
		N	2.4	1.8	4.2
		S	28.9	16.7	45.7
23-Mar	M	O	20.8	21.8	42.6
		N	15.1	16.1	31.2
		S	64.5	19.3	83.8
	H	O	67.2	28.7	95.9
		N	43.1	23.1	66.2
		S	1.2	6.3	7.5
	L	O	0.9	4.4	5.3
		N	1.1	6.2	7.4
		S	14.8	25.8	40.6
4-Apr	M	O	5.6	23.0	28.6
		N	10.2	35.2	45.3
		S	33.3	29.3	63.2
	H	O	46.6	38.7	84.0
		N	48.2	105.0	157.3
		S	2.3	14.6	16.9
	L	O	1.2	14.4	15.6
		N	2.0	20.2	22.2
		S	12.0	115.2	125.2
18-Apr	M	O	1.9	66.1	67.9
		N	3.7	78.1	81.2
		S	15.9	221.1	231.8
	H	O	21.9	182.7	187.9
		N	5.7	187.4	190.1
		S	1.0	26.4	27.4
	L	O	1.2	27.4	28.6
		N	1.3	19.6	20.9
		S	2.7	24.1	26.8
17-May	M	O	2.4	44.4	46.9
		N	6.4	18.5	24.9
		S	1.7	58.0	59.7
	H	O	2.0	70.5	72.5
		N	3.4	43.8	48.1
		E	<0.0001	<0.0001	<0.0001
		PD	0.03	0.82	0.54
		S	<0.0001	<0.0001	<0.0001
		R(E*S)	<0.0001	0.14	0.012
		PD*S	0.14	0.015	0.014
		E*PD	0.20	0.82	0.72
		E*S	<0.0001	<0.0001	<0.0001
		E*PD*S	0.85	0.48	0.43

Table 4.19. Yield (Y) (g m^{-2}), % grain N (Ng), and N grain content (Ngc) (g m^{-2}) LSMEANS for 8 soft red winter lines planted September (S), October (O), and November (N) under 0 kg N ha^{-1} (L), 101 kg N ha^{-1} (M), 168 kg N ha^{-1} (H) from the hill plot study Lexington, KY 2013.

		Y			Ng			Ngc		
Genotype		L	M	H	L	M	H	L	M	H
25R32	S	415.9	704.0	670.0	1.99	2.53	2.71	8.2	17.9	18.2
	O	65.3	174.6	292.4	2.35	2.46	2.68	1.5	4.4	7.7
	N	28.4	109.3	149.0	2.35	2.47	2.84	0.7	2.7	4.2
KY02C-1058-03	S	323.6	620.3	610.3	2.18	2.54	2.73	7.0	15.8	16.7
	O	82.3	197.3	144.8	2.27	2.60	2.71	1.9	4.9	3.9
	N	18.5	35.5	47.1	2.18	2.65	2.99	0.4	0.9	1.3
KY04C-1128-38-1-5	S	360.5	681.3	800.6	2.01	2.22	2.61	7.3	14.8	20.9
	O	65.3	187.4	198.7	2.45	2.28	2.73	1.6	4.3	5.3
	N	22.7	66.7	133.4	2.20	2.41	2.66	0.5	1.6	3.5
KY05C-1617-17-17-3	S	352.0	569.2	481.2	2.24	3.31	2.83	7.9	18.5	13.5
	O	56.8	195.9	285.3	2.35	2.48	2.66	1.3	5.0	7.4
	N	17.5	36.2	55.4	2.60	2.55	2.63	0.4	0.9	1.5
KY97C-1238-17-1	S	244.1	388.9	451.4	2.30	2.34	2.67	5.5	9.1	12.0
	O	86.6	211.5	141.9	2.22	2.50	2.68	1.9	5.3	3.8
	N	17.0	69.6	117.8	2.30	2.32	2.55	0.4	1.6	3.1
PEMBROKE	S	333.6	491.1	501.1	2.24	2.61	2.81	7.5	12.7	13.6
	O	80.9	177.4	249.8	2.41	2.40	2.67	2.0	4.2	6.6
	N	31.2	126.3	154.7	2.40	2.53	2.65	0.7	3.2	4.0
SHIRLEY	S	501.1	486.9	623.1	2.57	2.21	2.45	12.7	10.8	15.3
	O	110.7	292.4	259.8	2.25	2.38	2.67	2.5	7.0	6.9
	N	28.4	78.1	136.3	2.19	2.52	2.66	0.6	1.9	3.6
TRUMAN	S	448.5	638.7	667.1	2.78	2.70	2.72	12.6	17.2	18.1
	O	132.0	278.2	279.6	2.46	2.62	2.74	3.2	7.3	7.9
	N	36.9	141.9	168.9	2.71	2.77	2.67	0.9	3.9	4.5
$\bar{x}$		160.8	289.9	317.5	2.3	2.5	2.7	3.7	7.3	8.5
SE		33.0	45.4	45.6	0.04	0.05	0.02	0.79	1.2	1.2

Table 4.20. N uptake efficiency (NUpE), N utilization efficiency (NUtE) (g yield g^{-1} plant N), and N use efficiency (NUE) LSMEANS for 8 soft red winter lines planted September (S), October (O), and November (N) under 0 kg N ha^{-1} (L), 101 kg N ha^{-1} (M), 168 kg N ha^{-1} (H) from the hill plot study Lexington, KY 2013.

		NUpE			NUtE			NUE		
Genotype		L	M	H	L	M	H	L	M	H
25R32	S	1.93	1.46	0.98	40.2	31.7	31.4	78.1	45.7	30.3
	O	0.34	0.33	0.43	35.7	34.7	30.1	12.3	11.3	13.2
	N	0.14	0.21	0.24	37.5	33.6	28.0	5.3	7.1	6.7
KY02C-1058-03	S	1.76	1.28	0.99	34.5	31.5	28.2	60.8	40.2	27.6
	O	0.47	0.37	0.25	33.0	33.0	27.2	15.5	12.8	6.5
	N	0.10	0.09	0.12	36.9	25.5	22.1	3.5	2.3	2.1
KY04C-1128-38-1-5	S	1.67	1.18	1.18	40.8	37.2	30.7	67.7	44.2	36.2
	O	0.34	0.33	0.31	35.9	37.4	28.2	12.3	12.2	9.0
	N	0.16	0.13	0.20	35.6	33.2	29.0	4.3	4.3	6.0
KY05C-1617-17-17-3	S	2.23	1.49	0.90	30.4	25.7	24.1	66.1	36.9	21.7
	O	0.30	0.41	0.44	34.9	32.6	28.8	10.7	12.7	12.9
	N	0.10	0.13	0.10	25.2	26.0	25.9	3.3	2.3	2.5
KY97C-1238-17-1	S	1.39	0.78	0.82	33.6	33.2	24.8	45.9	25.2	20.4
	O	0.48	0.46	0.23	35.1	29.7	27.7	16.3	13.7	6.4
	N	0.09	0.14	0.20	33.7	31.6	26.7	3.2	4.5	5.3
PEMBROKE	S	1.75	1.01	0.72	35.1	31.9	30.1	62.7	31.9	22.6
	O	0.45	0.32	0.36	34.7	35.7	31.3	15.2	11.5	11.3
	N	0.17	0.27	0.23	33.7	31.0	29.7	5.9	8.2	7.0
SHIRLEY	S	2.83	0.96	0.98	34.1	33.3	28.6	94.1	31.6	28.1
	O	0.59	0.55	0.39	35.7	34.5	29.8	20.8	19.0	11.7
	N	0.14	0.16	0.21	36.7	31.7	28.9	5.3	5.1	6.2
TRUMAN	S	3.14	1.47	1.04	28.8	28.6	29.2	84.2	41.4	30.1
	O	0.80	0.51	0.43	30.7	30.7	30.6	24.8	18.1	12.6
	N	0.21	0.35	0.27	32.2	28.4	28.1	6.9	9.2	7.6
$\bar{x}$		0.90	0.60	0.50	34.4	31.8	28.3	30.2	18.8	14.3
SE		0.19	0.10	0.07	0.68	0.66	0.47	6.2	2.9	2.1

Figure 4.1. Estimated average plant available N (ppm) from resin bag sampling under the 0 kg N ha^{-1} environment analyzed for each planting date (September (LS), October (LO), and November (LN)) from the hill plot study Lexington, KY 2013.

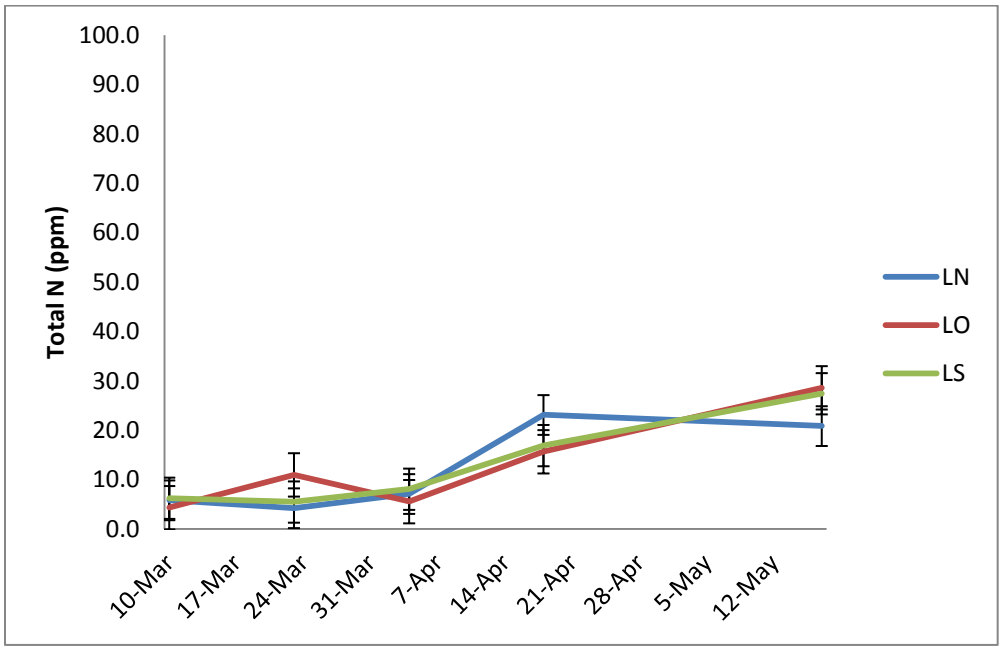


Figure 4.2. Estimated average plant available N (ppm) from resin bag sampling under the 101 kg N ha^{-1} environment analyzed for each planting date (September (MS), October (MO), and November (MN)) from the hill plot study Lexington, KY 2013.

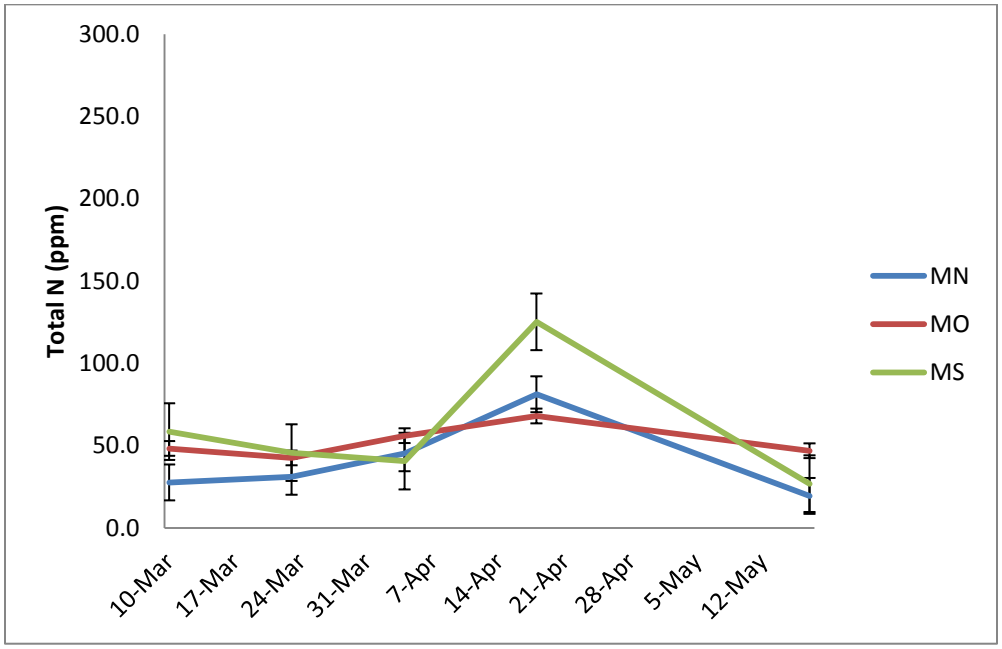
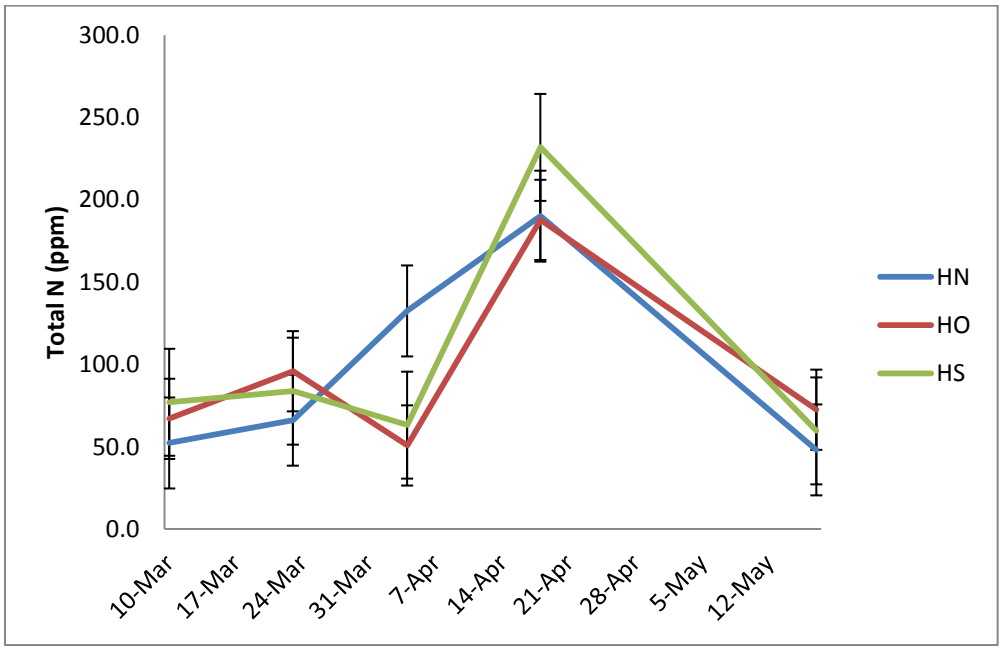


Figure 4.3. Estimated average plant available N (ppm) from resin bag sampling under the 168 kg N ha^{-1} environment analyzed for each planting date (September (HS), October (HO), and November (HN)) from the hill plot study Lexington, KY 2013.



Chapter 5

Selecting for Nitrogen-Use Efficient Soft Red Winter Wheat Lines Under High and Low Nitrogen Environments

Introduction

Winter wheat (*Triticuum aestivum* L.) is an important component of the national and global food supply. In recent years, evidence has shown that yields have not increased significantly (Hatfield and Prueger, 2004; Muurinen *et al.*, 2007). As human population growth continues, worldwide demand for wheat will continue to increase. Supplying food to future populations, while producing agricultural crops sustainably, is becoming an increasing problem. Nitrogen-use efficient crop varieties may be a good option to ensure sustainability in agricultural systems and meet future consumer demand. Adding excess nitrogen (N) to crops has been shown to have adverse environmental impacts, such as eutrophication of freshwater and marine ecosystems that occur when high quantities of N fertilizer are added to soil and then washed into the stream through runoff (Sieling *et al*, 2009). Reduction in fertilizer use, on the other hand, could also decrease crop yield and quality if the plant experiences N deficiency (Cassman *et al.,* 2003). Therefore, great interest has been focused on crop varieties with high nitrogen use-efficiency (NUE) because these plants would be expected to minimize environmental and production costs associated with addition of excess N to agricultural systems. As a result, selecting and developing NUE crops has gained momentum among breeders. Nitrogen use efficincy is described as grain yield produced per unit soil N supply (soil N and fertilizer N) (Moll *et al.* 1982). Nitrogen use efficiency consists of two components, N uptake efficiency (NUpE) (ability of the plant to take up soil N) and N utilization efficiency (NUtE) (ability of the plant to generate yield from N accumulated in vegetative tissue) (Moll *et al.*, 1982; Nyikako *et al.*, 2014). Nitrogen uptake efficiency can be calculated by dividing total plant N by the soil N supply and NUtE (kg yield kg^{-1} plant N) can be measured by dividing yield by the total plant N. Total plant N (kg ha^{-1}) is the

amount of N in the aboveground material at maturity (grain N (kg ha^{-1}) (yield * % N grain) + N content in straw (kg ha^{-1}) (biomass * % N straw) (Moll *et al.*, 1982).

Increases in NUE are dependent on NUpE and NUtE. Most wheat breeding trials are conducted under high N environments. This means plant breeders select varieties that perform well under these conditions. Many studies have shown genetic variation in NUtE and NUpE (Van Sanford and MacKown, 1986; Ortiz-Monstarerio *et al.* 1997, Foulkes *et al.* 1998, Muurinen *et al.* 2006; Barraclough *et al.*, 2010; Gaju *et al.*, 2011; Gaju *et al.*, 2014). These studies illustrated an interaction between genotype and N supply on NUE, thus influencing NUpE and NUtE (Ortiz-Monstarerio *et al.* 1997, Foulkes *et al.* 1998, Muurinen *et al.* 2006; Barraclough *et al.*, 2010; Gaju *et al.*, 2011; Gaju *et al.*, 2014). Therefore, genotypes selected for high yield under high N environments may not produce high yields under low N environments. As a result, breeding and selection in low N environments may be needed to appropriately identify varieties with sufficient yield and NUE. A knowledge of which traits are important for NUE under these N conditions is necessary to identify efficient cultivars (Brancourt-Hulmel *et al.*, 2005). For instance, some studies have shown a relation between final grain N and NUpE in both high and low N environments. Measuring total plant uptake at anthesis and maturity can be useful trait to estimate N partitioning. At anthesis, measuring total N uptake can give insight into growth of yield generating leaves, floret fertility, amount of stem reserves, and creation of deep root system. Nitrogen accumulation at this developmental stage is reliant on the extent of the rooting system and N availability. After anthesis, biomass partitioning can indicate preferred strategies in N storage and translocation (Cox *et al.* 1986; Swain *et al.*, 2014). Measuring total N uptake at maturity provides information on the translocation efficiency of N from the biomass to the grain. Wheat genotypes that have superior N uptake, N storage, and N translocation capabilities will allow for further gains in NUE, along with genotypes that posses the "stay green" trait which hinders senescence and allows for a longer grain filling period through the continuation of N uptake and translocation (Bogard *et al.* 2011; Swain *et al.*, 2014).

Estimating NUE and traits related to NUE can be time and resource consuming. However, more rapid and efficient selection for high yielding and stress tolerant plants

may be possible through high-throughput phenotyping. Canopy spectral reflectance (CSR) devices can be utilized to implement high-throughput phenotyping for complex traits such as N concentration associated with NUE (Li *et al*, 2014). Canopy spectral reflectance devices measure the amount of light reflected/absorbed by the plant's canopy surface. Genotypic variation and environmental stress can affect the amount of light reflected. Thus, the use of CSR in selection for NUE may be a rapid and inexpensive option (Raun *et al.*, 2001). A CSR index, such as the normalized difference vegetative index (NDVI), has been shown to have high correlations with wheat grain yield, biomass and N concentration (Ma *et al.*, 1996; Raun *et al.*, 2001; Crain *et al.*, 2012). The CSR estimates of biomass and N content can be used to estimate NUpE and NUtE. The relationship of CSR with biomass is of great interest, since biomass is an essential element related to NUE and yield (Crain *et al.*, 2012). Therefore the objectives this study were to: identify high NUE genotypes grown under low and high N environments at multiple locations, estimate the value of the canopy spectral reflectance device (Crop Circle) as a high-throughput phenotyping method for NUE selection in wheat, and determine traits associated with NUE.

Materials and Methods

Site description and Experimental Design

The study was planted at two locations, University of Kentucky Spindletop Research Farm in Lexington, KY ($38^0$7'37.81''N, $84^0$29 44.85'' W) and West Kentucky Research and Educational Center in Princeton, KY ($37^0$6'7.37'' N, $87^0$52'13.62''W). The soil at the Lexington site was characterized by Maury silt loam [fine, mixed, semiactive, mesic Typic Paleudalfs] soil. The soil at the Princeton site was characterized by Crider silt loam [fine-silty, mixed, active, mesic, Typic Paleudalfs] soil. The study consisted of 56 winter wheat genotypes. These genotypes comprised one block of the TCAP eastern elite wheat panel (triticaeacap.org). These breeding lines were chosen because this block of material was well adapted to Kentucky, containing breeding lines from KY, IN, and IL which represented a sample of the diversity contained within the UK wheat breeding program. These breeding lines were thought to vary in grain protein

content and nitrogen-use efficiency (NUE) because differences were observed among the lines for other traits like heading date and height. The 56 soft red winter wheat lines were planted in a randomized block design in 0 kg N ha^{-1} and 112 kg N ha^{-1} environments at two locations, Princeton, KY and Spindletop farm in Lexington, KY. The experimental unit was a single 6-row yield plot 3.3 m in length, 1.2 m wide. There were two replications for each treatment at each location. In the 112 kg N ha^{-1} treatment. Nitrogen was applied in a 34 kg N ha^{-1} and 78 kg N ha^{-1} split, 13 March 2014 and 9 April 2014 in Princeton and 21 March 2014 and 17 April 2014 in Lexington respectively.

Field Sampling and Data Collection

Soil Sampling

Soil samples were taken prior to N application at each location along with vegetative material samples from 10 genotypes. These 10 genotypes differed in other agronomic traits such as heading date and height, thus these plants were expected to represent the variation in vegetative N concentration prior to N fertilizer application. At both locations, twenty soil cores were taken from each N environment for each replication to a depth of 30.48 cm using a 1.6 cm diameter soil probe. The cores collected from a particular N environment and location were mixed by hand in a plastic bucket, air dried on paper bag, and placed in a paper soil bag.

Agronomic Traits and N Sampling

Nitrogen status of each genotype within each environment at both locations was measured at Feekes 10 (boot stage) using the hand held canopy spectral reflectance device the Crop Circle. The device was held 56 cm above the plot and walked along the length of the plot at a steady pace. The device measured normalized difference vegetative index (NDVI) for each plot. Normalized difference vegetative index is calculated by the machine using the following formula: (780nm - 680nm/780nm + 680nm). For each plot, heading date and anthesis date was recorded. Heading date was determined when 50% of the spikes in the plot had emerged from the boot. Anthesis date was recorded when 50% of the plants in a plot were flowering. Plot length and plot height were measured at the soft dough stage which is equivalent to physiological

maturity, defined as when maximum dry matter accumulation has occurred and kernel has turned a buff color. Within each plot at each location, a 30.48 cm length of row was collected at anthesis and harvest maturity for whole plant N analysis. Harvest maturity was determined when grain was hard and could not be split by thumbnail. Plots were combine harvested in Princeton and Lexington on 24 June and 2 July 2014, respectively. Harvested grain from each plot from both locations was taken in harvest bags to measure yield, moisture content, and test weight using the GAC.

Data processing and Analysis

Soil N analysis

Soil samples collected within each treatment and location were extracted for ammonium and nitrate using the KCl method. Ten grams of soil from each sample was shaken for 30 minutes in 25 mL of 2 mol KCl (150 g KCl per 1000 mL of deionized (DI) water) at 200 rpm. Then, 1 mL liquid extractant from each sample was pippetted (a new pipette head was used per sample) into cluster tubes, recording the order samples were arranged in the cluster tube box so that the data could be associated with the correct sample once analysis was complete. The cluster tube box with samples was centrifuged for 27 minutes. Then, 15 µL of standards and samples was pippetted into the wells of two microplates, one for nitrate analysis and one for ammonium analysis.

To prepare the nitrate microplate for analysis, 200 µL of pH 8.5 ammonium buffer was added to each nitrate microplate well. A copperized cadmium reductor was placed into each well of the microplate and shaken for 60 minutes on a titer plate shaker to convert nitrate to nitrite (Nydhal, 1976; Crutchfield and Grove, 2011). The Griess reaction was used to colormetrically measure the nitrite concentration within each sample. To induce the Griess reaction, 60 µL of Griess reagent (4 mL of 1.0 % sulfanilamide in 3 N hydrochloric acid and 4 mL of 0.1 % N-(1-Naphthyl)) was added to each well of the microplate (Griess, 1858; Crutchfield and Grove, 2011). The microplate was shaken for additional 5 minutes. The microplate was inserted into the Microplate Versa Max Analyzer and nitrite levels within each well were read at 542 nm (Henriksen and Selmer-Olsen, 1970; Crutchfield and Grove, 2011).

The ammonium microplate was colormetrically measured using a modified Berthelot reaction. The Berthelot reaction was induced by inserting 100 µL of sodium hydroxide-hypochlorite and 100 µL of phenol-nitroprusside into each microplate well (Berthelot, 1859; Chaney and Marbach, 1962; Weatherburn, 1967; Ngo *et al.*, 1982). Then the microplate was shaken on a titer plate shaker for 45 minutes. Afterwards, the microplate was inserted into the Microplate Versa Max Analyzer and ammonium levels were measured at 630 nm. Prior to N application soil N determined to be 56.3 kg N ha^{-1} at PRN and 42.3 kg N ha^{-1} at LEX in the low N environment. At the high N environment, residual soil N was determined to be 42.1 kg N ha^{-1} at PRN and 40.6 kg N ha^{-1} at LEX. The total N supply after N application (101 kg N ha^{-1}) under the high N environment was 154.6 kg N ha^{-1} at PRN and 152.6 kg N ha^{-1} at LEX.

Measuring N traits

Pre-N vegetative material was air dried in the greenhouse, ground to a fine powder using a UDY cyclone grinder, and analyzed by the FlashEA 1112 combustion analyzer to measure percent N concentration. The vegetative samples collected from each plot at anthesis and maturity were treated similarly to Pre-N vegetative samples, but were analyzed for % N content in aboveground vegetative tissue using a Near-Infrared Reflectance (NIR) (Perten instrument DA7200) device. Whole grain samples from each plot and location were taken from the harvested grain and measured for grain protein using the NIR. Grain protein was converted to % grain N by using the conversion factor for grain, 6.25. Total plant N uptake (kg ha^{-1}) was determined by summing plant N in grain (kg ha^{-1}) (yield*%N) and vegetative tissue at maturity (kg ha^{-1})(biomass *% vegetative N at maturity). Post-anthesis N uptake (kg ha^{-1}) was calculated by subtracting the N in vegetative tissue at anthesis (kg ha^{-1}) (anthesis biomass*% vegetative N at anthesis) by total plant N. Nitrogen-use efficiency (NUE) and NUE components (nitrogen uptake efficiency (NUpE) and nitrogen utilization efficiency (NUtE)) calculated using the following formulas: NUpE= Total plant N/ soil N (Pre-N soil N and fertilizer N), NUtE (kg yield kg^{-1} plant N) =yield/total plant N, NUE= (NUpE) (NUtE).

Statistical Analysis

Analysis of variance (ANOVA) was performed using the General Linear Models procedure (Proc GLM; SAS 2002) to determine genotype and treatment effects across locations and N environments. The model used was:

$Y_{ijkl} = \mu + LOC_i + R(LOC)_{ij} + ENV_l + G_k + ENV_l*G_k + LOC_i*G_k + LOC_i*ENV_l + LOC_i*ENV_l*G_k + E_{ijkl}$

Where: Y_{ijkl} = the observation in the *k*th genotype in the *j*th rep in the *i*th location and *l*th N environment, μ = the overall mean, LOC_i =the effect of the *i*th location, $R(LOC)_{ij}$ = the effect of *j*th rep within the *i*th location, ENV_l = the effect of the *l*th N environment, G_k = the effect of the *k*th genotype, $ENV_l * G_k$ = the effect of the interaction of the *l*th N environment with the *k*th genotype, LOC_i*G_k= the effect of the interaction of the *i*th location with the *k*th genotype, LOC_i*ENV_l=the interaction effect of the *i*th location and *l*th N environment, $ENV_l*LOC_i*G_k$ = the effect of the interaction of the *l*th N environment and *i*th location with the *k*th genotype, E_{ijkl} = the residual error. Least square means (LSMEANS) were calculated to estimate differences among locations and treatments.

Broad sense heritability of N traits and agronomic traits was estimated on an entry mean basis using the following model:

$Y_{ijk} = \mu + G_k + R(ENV)_{ij} + ENV_i * G_j + E_{ijk}$;

where: Yij = the observation in the *k*th genotype in the *j*th rep in the *i*th environment, μ = the overall mean, Gj = the effect of the *k*th genotype, R(ENV)ij = the effect of *j*th rep within *i*th environment, ENVi * Gj = the effect of the interaction of the *i*th environment with the *k*th genotype, Eij = the residual error.

Agronomic and N trait data was analyzed using the General Linear Models procedure (Proc GLM; SAS 2002). Genotypic and phenotypic variances were estimated from the expected mean squares (EMS) and heritability estimates were computed as:

$h^2 = Vg/Vp$;

where h^2 = heritability, Vg = genotypic variance, Vp = phenotypic variance.

Confidence intervals (90 %) were calculated after Knapp *et al.* (1985) as:

UL= 1- [MS3/MS2 * FUL (.10, v1 and v2 df)]-1

LL= 1- [MS3/MS2 * FLL (.90, v1 and v2 df)]-1

where: UL = upper limit of the confidence interval, MS3 = entry mean square, MS2 = residual mean square, FUL and FLL = F value for the upper and lower limits calculated using the FINV function of Microsoft Excel (2007).

PROC CORR (SAS 2002) was used to analyze the relationship among traits on an entry mean basis.

TASSEL

All entries in the mapping panel were genotyped with the 9K Illumina SNP chip to identify single nucleotide polymorphisms (SNP) associated with the traits measured during the course of the study. TASSEL (http://www.maizegenetics.net) software was used to carry out association mapping. The Q+K method was implemented as a mixed linear model to determine associations of the N and agronomic traits measured with QTL markers. The statistical model used was described as:

Y= Xb+ Zu+e

where y is the vector of observations; b is an unknown vector containing fixed effects including genetic marker and population structure (Q); u is an unknown vector of random additive genetic effects from multiple background QTL for individuals or lines; X and Z are the known design matrices; and e is the unobserved vector of random residuals (Bradbury *et al.* 2007).

Results and Discussion

Agronomic traits

The analysis of variance (ANOVA) determined the significance of each trait at the N environment (E), location (L), genotype (G), genotype*treatment (G*T),

genotype*location (G*L), location*environment (L*E) and genotype*treatment*location (G*T*L) levels (Table 5.1). Across locations, under low N, development was quickened as indicated by earlier heading (HD) and anthesis dates (AD) than under the high N treatment. All agronomic traits other than test weight were significantly different between each location (Table 5.1). All agronomic traits except grain protein were significantly different between N environments. A single year study examining differences in uptake efficiency and N partitioning under different N environments and locations, also found no significant difference in grain protein between low and high N environments (Barraclough *et al*., 2014). Possibly the differences in grain protein would have been observed over multiple years.

Genotypic variation was observed for traits such as heading date, anthesis date, height, yield, and grain protein. However, there was no genotype*N environment (G*E) or genotype*location*N environment (G*L*E) interaction except for heading date (Table 5.1). At LEX, heading date did not differ significantly between the two N treatments (Table 5.1). There is a possiblity that development lagged at LEX due to the very harsh winter, thus causing heading date to be uneven making the trait difficult to read. Agronomic measurements such as yield, biomass, test weight, and harvest index (HI) were lower under low N than high N as expected and were significantly different across locations and treatments, except test weight which differed significantly only between N environments. Genotypic variation for yield was greater under low N than high N, <0.0001 and 0.05 respectively (Tables 5.2 and 5.3), indicating that yield may be better selected for under N limiting environments.

There existed genotype*location (G*L) interactions for traits such as HD, AD, HI and yield. Heading date and anthesis date were earlier under the PRN location and earlier under the low N environment. Both anthesis and maturity harvest biomass measurements were higher at PRN for both treatments than at LEX. However, yields under both N treatments were lower at PRN than LEX (Table 5.1). It is possible that when N was applied at PRN the ground was wet and thus dentrification may have occurred. There may have been sufficient N available for biomass production but less N was available for grain production at PRN. Percent soil moisture at 10.16 cm depth at LEX and PRN were similar 37% and 34.1% respectively. However, at 101.6 cm depth, soil moisture at LEX

was much lower than at PRN, 47% and 66.6% respectively (http://wwwagwx.ca.uky.edu/), indicating a larger loss of N due to denitrification at PRN than LEX. The top yielding varieties in each environment are as follows: LEX low N: KY93C-1238-17-1 (3375.5 kg ha^{-1}), LEX high N: IL-20728 (6227.8 kg ha^{-1}), PRN low N: IL07-20743 (2886.4 kg ha^{-1}), PRN high N: KY03C-1002-02 (5201 kg ha^{-1}). The most stable genotypes across environments in terms of yield are represented in Table 5.4, the most stable being KY93C-1238-17-1 and IL01-11934. These may be good candidates to be incorporated into breeding programs to develop high yielding adapted to Kentucky's environment.

N traits

All N traits except vegetative biomass at maturity were significantly different between N environments, except N concentration at maturity. All N traits except for PANU and NDVI were significantly different across locations. Traits such as N in vegetative biomass at maturity, NHI, NDVI, NUE, NUtE, and NUpE were all significantly different among the genotypes; NUpE and NHI at ($p<0.05$) and the rest at ($p<0.01$) (Table 5.5). There was no G*L*E interaction for the N traits measured (Table 5.5). There was a G*E interaction for N in vegetative biomass at maturity ($p<0.01$) and NUtE ($p<0.05$). G*L interaction for NUE and NUpE was significant at $p<0.01$. The low N environment had lower N in the biomass at anthesis and maturity, lower grain N content, and total plant N than the high N treatment. The high N environment had lower NUpE, NUtE, and NUE than the low N environment, but this is an effect of N supply (Table 5.5). Nitrogen uptake efficiency, NUtE, and NUE increase under low N supply, since the N supply was small, the plants were able to take up most of what was available.

At Lexington, all N traits ($p<0.01$) except vegetative N content at maturity were significantly different between N environments. Genotypic variation was significant for N in vegetative biomass at maturity, PANU, NHI, NUE, NUtE, NUpE ($p<0.01$). Only NUtE ($p<0.01$) and NUE ($p<0.05$) had a G*E interaction at LEX. At PRN, all N traits were significantly different between N environments except N concentration at anthesis. There was no significant genotypic variation for the N traits other than NDVI ($p<0.05$), nor was there G*E interaction for N traits at PRN. NUtE was higher at PRN (Table 5.5).

Overall, NUE was higher at LEX, along with yield, grain N content, total plant N, and NUpE (Table 5.1 and 5.5). N concentration at anthesis and maturity was lower at the PRN location for both N environments, along with vegetative N in the biomass at anthesis and maturity (Table 5.5). The lack of genotypic variation among the N traits and lower N vegetative measurements may be possibly related to the loss of N from dentrification at PRN. Lower N supply at PRN meant less uptake and thus lower N concentration at anthesis and maturity, N in vegetative biomass, and total plant N; resulting in lower yield and grain N, and higher NUtE.

Uptake efficiency ranged from 0.43-1.53 across N environments and locations. Utilization efficiency ranged from 41.7-64.7 kg yield kg^{-1} plant N. Nitrogen use efficiency ranged from 32-80. At LEX, uptake efficiency had a stronger positive correlation to yield than utilization efficiency in each N treatment, low N: 0.79 ($p<0.01$), 0.56 ($p<0.01$) and high N: 0.81 ($p<0.01$), 0.46 ($p<0.01$) respectively. A similar trend was seen at PRN under both low N: 0.85 ($p<0.01$), 0.60 ($p<0.01$) and high N: 0.72 ($p<0.01$), 0.44 ($p<0.01$), respectively (Tables 5.6 and 5.7). Grain N (kg ha^{-1}) also had a very strong positive correlation to uptake efficiency over utilization efficiency under both N treatments across locations (Tables 5.6 and 5.7). Nitrogen uptake efficiency was more strongly correlated to yield and N grain across locations and N environments. Therefore, NUpE or total plant N may be more important than NUtE in contributing to yield and N grain content. Thus, selecting for NUpE and developing strategies to identify traits related to uptake may be a good method for increasing overall NUE, yield, and grain N in our region. In the low N environment, genotypic variation was on average higher than in the high N environment (Tables 5.8 and 5.9). Nitrogen uptake efficiency, NUE, NUtE, and total plant N were associated with genotypic variation under low N environment (Table 5.8). Genotypic variation was higher for NUtE in the high N environment than low N environment, ($p<0.0001$ and $p<0.05$, respectively; Table 5.7). In low N, genotypic variation for NUpE and NUE was greater than in the high N environment, NUpE: $P<0.01$ and 0.63, respectively; NUE: $p<0.0001$ and $p<0.05$, respectively (Tables 5.5 and 5.7). Under the low N environment, NUpE was associated with more of the genetic variation in NUE, while under the high N environment more of the genetic association in NUE was related to NUtE.

Results from this study showed that NUpE may be more important to genotypic performance and NUE than NUtE. Yield, protein, grain N, total plant N, biomass at maturity, and N in vegetative biomass at maturity were more closely related to NUpE than NUtE across study sites and N environments. Genotypes that had the most stable yields across N environments and locations tended to have the most stable NUpE as well. A study done in Kentucky using 25 soft red winter wheat cultivars grown under high N also found strong significant correlations to yield and protein with NUpE, 0.70 and 0.80, respectively (Van Sanford and MacKown, 1986). In the present study, correlations between NUpE and protein under high N were not as strong, but were still significant. Also, NUpE and NUE had stronger genetic variation under the low N environment than high N environment across locations. Nitrogen uptake efficiency may have a stronger association with NUE in N limiting environments. However, in high N, genetic variation was much greater for NUtE than NUpE. Thus, NUtE may have a greater association with NUE in environments with sufficient N. Other studies across the globe have also indicated that NUpE is more closely associated with NUE under various N environments. Other studies have also indicated a greater portion of genetic variation in NUE was explained by NUpE under low N environments and NUtE in high N environments. These studies include an experiment done in Mexico using 10 spring wheat cultivars (Ortiz-Monasterio *et al.*, 1997), 20 winter wheat varieties grown under multiple N environments in France (LeGouis *et al.*, 2000) and 40 spring wheat cultivars grown in Finland (Muurinen *et al.*, 2006). However, genotypic variation for NUtE was significant under the low N environment ($p<0.05$), but not as significant as NUpE ($p<0.01$). Even though NUpE was more strongly associated with yield, N grain content, and NUE; NUtE did have significant correlations to these traits across locations and N environments. Therefore, plant response to N limitation depends on both NUpE and NUtE in low N environments, but more emphasis is placed on NUpE. The authors of a similar study using 16 wheat genotypes planted under multiple N environments and locations came to a similar conclusion (Gaju *et al.*, 2011). The researchers found that both NUtE and NUpE played a role in grain yield response amongst the cultivars under N limited environments.

The mechanisms associated with variation in NUpE and NUtE among the cultivars were not evident from this study. There were significant associations between

height and NUtE and NUpE LEX high N environment and PRN location, but these correlations were weak (Tables 5.6 and 5.7). Some studies have suggested that NHI could be used to select for N use efficient genotypes. Nitrogen harvest index did have a strong correlation to NUtE across N environments and locations, but had weak correlations to yield, grain N, NUE, and NUpE (Tables 5.6 and 5.7). Often, there were genotypes with high NHI, but low NUE or low NHI and high NUE (Tables 5.10 and 5.11). Therefore, using NHI to select for NUE genotypes may not be efficient in either low or high N environments. From the association analysis in TASSEL, no SNP's were found to be significant across N environments for measured N traits according to the Bonferroni test adjustment, $p < 4.7*10^{-7}$ for $p < 0.01$ and $p < 2.4*10^{-6}$ for $p < 0.05$ (Tables 5.12, 5.13, 5.14, 5.15). Even though there were was no relationship between physiological traits and N parameters in this study, some studies have found evidence that these associations do exist. For example, previous studies have shown a strong relationship between NUpE and anthesis date, later anthesis date resulting in increased NUpE (Laperche *et al.*, 2006). Also, root morphology likely influences NUpE, especially under limiting N supply. Development of new roots is necessary for the plant to make use of resources in unexplored soil and root morphology may vary among genotypes, and thus root traits are likely important in determining crop NUpE and overall NUE (Baresel *et al.*, 2008). Foulkes *et al.* (2004) suggested that deep rooting systems may be favored by later anthesis date, thus affecting NUpE. Therefore, examining root structure of cultivars under low N environments may help define traits associated with NUpE and overall NUE.

Post-anthesis N uptake

Post-anthesis N uptake (PANU) was significantly different between N treatments (Table 5.5). There was no significant genotypic variation for PANU under the low or high N environment though genotypic variation in PANU increased under high N. Post-anthesis N uptake was much higher in the high N (LEX: 21.8 and PRN: 23.6 kg ha^{-1}) treatment than low N treatment (LEX: .3 and PRN: -2.3 kg ha^{-1}) at both locations (Table 5.5). Post-anthesis N uptake also had a strong positive correlation to yield under the high N environment at each location (Tables 5.6 and 5.7). The correlation between PANU and

yield in the low N environment was positive but weaker, possibly because greater N supply under the high N environment allowed for more PANU (Table 5.6). The plants in the low N environment were stressed and had accelerated development, as indicated by earlier heading date and anthesis date (Table 5.1). Thus, there was less opportunity for PANU (Table 5.1 and 5.5). Post-anthesis N uptake had a significant positive correlation to N concentration at maturity at LEX. At PRN, PANU was positively correlated to N concentration at maturity but was not significant. PANU was also strongly correlated to total plant N and NUpE at PRN and LEX under high N. Grain N (kg ha^{-1}) also had a strong positive correlation with PANU at the high N environments, LEX: 0.96 ($p<0.01$), PRN: 0.73 ($p<0.01$) (Table 5.8). Therefore, PANU may be advantageous to grain N (kg ha^{-1}), yield and overall NUE, especially in N sufficient environments by increasing NUpE and total plant N. However, PANU had a low estimate of heritability, 0.28 (Table 5.16). Therefore, selecting for traits associated with PANU could be a possible aim for breeding improved yield and N grain content performance in high N environments. Post-anthesis N uptake was negatively correlated to NUtE across environments (Tables 5.6 and 5.7), though the correlations were weak. This suggests that there may have been some luxury consumption of soil N among some of the wheat cultivars. Other wheat studies have also found a negative correlation between NUtE and PANU, suggesting that the some plants may have taken up more N than needed (Gaju *et al.*, 2011 and 2014).

Canopy Spectral Reflectance

The ability of the normalized difference vegetative index (NDVI) to identify genotypes with high NUE was tested using the Crop Circle. Normalized difference vegetative index was significantly different between N environments, but not locations. There was no significant genotype by environment interaction (G*E, G*L, G*L*E) for NDVI (Table 5.5). Genotypic variation was significant under the high N environment, but not the low N environment. The normailized difference vegetative index was positively correlated to yield and NUE across environments, LEX low N: 0.05 and LEX high N: 0.39 ($p<0.01$); PRN low N: 0.36 ($p<0.01$) and PRN high N: 0.44 ($p<0.01$). There was a significant positive correlation between NDVI and NUpE in the PRN low N (0.30, $p<0.01$) and LEX high N (0.50, $p<0.01$) and NUtE under PRN high N (0.39, $p<0.01$)

(Table 5.6 and 5.7). There was no significant correlation between NDVI in the LEX 0N environment for NUE or its components (Table 5.6). Even though there was a significant correlation between NDVI and NUE components in almost all environments, the correlation was weak. Using NDVI as a method of selecting for NUE may not be a viable option in soft red winter wheat.

Implications for Breeding

Selection for NUE in the U.S. is mainly conducted under high N environments. However, the present study indicated that genetic variation for yield, grain N, and NUE was higher in the low N environment. Under the low N environment, genetic variation was highest for NUpE as well. Plant performance for these traits may be masked in high N environments. Therefore, selection for these traits may be done more efficiently under N limiting environments. Genetic variation was greatest for NUtE and PANU in the high N environment, and thus selection for these traits may be more efficient under this environment. However, NUtE did have significant genotypic variation in the low N environment. Therefore, selection for genotypes with both adequate NUtE and NUpE may be possible under low N environments. Lines that have high NUtE in high N environments and high NUpE in low N environments or have both high NUpE and NUtE under low N environments may be good candidates for further development of N use efficient genotypes. For example, from the present study, genotype KY02C-1121-75 had the most stable NUpE across locations under low N, ranked 3 at LEX and 7 at PRN. This genotype also had very high NUtE across locations in high N, ranked 1 at LEX and 6 at PRN (Table 5.17). The genotype KY02C-1121-75 was also one of the most stable lines in terms of yield (Table 5.2). Therefore, this line could be used in breeding programs to develop N use efficient varieties; other high yielding lines that acted similarly to KY02C-1121-75, included IL01-11934 and IL07-20743.

However, not all high yielding lines were efficient in both NUpE and NUtE. For instance, K93C-1238-17-1 and KY03C-2314-08, were not stable in NUpE in low N environments, but were stable in NUtE across N environments and locations (Table 5.17). KY06C-1003-139-8-3 was stable in NUpE under low N, but not NUtE under high N environments. Selecting for yield alone does not necessarily mean a breeder is

selecting for improved NUpE and NUtE. The goal is to develop lines that have a high efficiency to take up soil N and that have a high capacity to utilize the N these plants have taken up to generate yield, thus increasing overall NUE. Identifying easily quantifiable traits related to NUE would be very beneficial to breeding programs. However, traits associated with NUE are also affected by environmental conditions. Many of the N traits, including NUpE and NUtE, had low heritability estimates (Table 5.16). To gain further insight into the mechanisms controlling NUE, other tools such as ecophysiological models could be quite useful (Dresbøll and Thorup-Kristensen, 2014). Directed improvement of NUE through identification of QTL's using molecular methods and association mapping could potentially amplify development of high performance NUE cultivars, as has been reported in oilseed rape (Bouchet *et al.*, 2014) or barley (Kindu *et al.*, 2014).

Table 5.1. LSMEANS for agronomic traits in each N environment from the N study Lexington (LEX) and Princeton (PRN), KY 0 (L) and 112 (H) kg N ha^{-1} N environment. Level of significance from ANOVA is shown for each trait. Rep (R), N environment (E), Location (L), genotype (G).

Environment	Heading date (May)	Anthesis date (May)	Height (cm)	Biomass anthesis (kg ha^{-1})	Biomass maturity (kg ha^{-1})	% Grain moisture	Test weight (kg hL^{-1})	Yield (kg ha^{-1})	HI (%)	% Grain protein
PRN L	6.2	10.1	68.1	3831.5	2158.3	21.5	63.3	2371.5	53.0	9.92
PRN H	8.3	12.4	85.8	4405.7	2998.4	21.9	63.4	4559.3	60.4	9.74
LEX L	13.2	14.7	68.6	3488.9	2164.2	14.5	68.1	2673.7	55.6	9.52
LEX H	13.9	15.7	76.6	3635.4	2782.0	14.4	70.0	4740.9	62.9	9.81
L	<0.0001	<0.0001	<0.0001	<0.0001	0.02	<0.0001	0.15	<0.0001	<0.0001	0.001
R(L)	<0.0001	0.0002	0.11	0.03	0.38	<0.0001	0.11	0.001	0.25	0.99
E	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	0.24
G	0.002	<0.0001	<0.0001	0.15	0.29	0.93	0.80	<0.0001	0.33	<0.0001
G*L	<0.0001	<0.0001	0.81	0.10	0.07	0.05	0.73	0.02	0.03	0.17
G*E	0.02	0.81	0.41	0.61	0.19	0.36	0.84	0.90	0.57	0.18
L*E	<0.0001	<0.0001	<0.0001	<0.0001	0.02	<0.0001	0.18	0.21	0.47	<0.0001
G*L*E	0.002	0.06	0.36	0.21	0.39	0.30	0.56	0.96	0.86	0.89

Table 5.2. LSMEANS for agronomic traits in each N environment from the N study Lexington (LEX) and Princeton (PRN), KY 0 (L) kg N ha^{-1} environment. Level of significance from ANOVA is shown for each trait. N treatment (E), location (L), genotype (G). Harvest index (HI).

Loc	Heading date (May)	Anthesis date (May)	Height (cm)	Biomass anthesis (kg ha^{-1})	Biomass maturity (kg ha^{-1})	% Grain moisture	Test weight (kg hL^{-1})	Yield (kg ha^{-1})	HI (%)	% Grain protein
LEX L	13.2	14.7	68.6	3488.9	2164.2	14.9	69.3	2673.7	55.6	9.52
PRN L	6.2	10.1	68.1	3831.5	2158.3	21.5	69.8	2371.5	53.0	9.92
L	<0.0001	<0.0001	0.49	0.0001	0.94	<0.0001	0.04	<0.0001	0.002	<0.0001
R(L)	0.003	0.24	0.51	0.03	0.66	<0.0001	0.54	0.08	0.58	0.71
G	0.14	<0.0001	<0.0001	0.35	0.28	0.77	0.38	<0.0001	0.77	0.0003
G*L	0.01	<0.0001	0.38	0.11	0.09	0.06	0.27	0.002	0.23	0.89

Table 5.3. LSMEANS for agronomic traits in each environment from the N study Lexington (LEX) and Princeton (PRN), KY 112 (H) kg N ha^{-1} environment. Level of significance from ANOVA is shown for each trait. Rep (R), location (L), genotype (G). Harvest index (HI).

Loc	Heading date (May)	Anthesis date (May)	Height (cm)	Biomass anthesis (kg ha^{-1})	Biomass maturity (kg ha^{-1})	% Grain moisture	Test weight (kg hL^{-1})	Yield (kg ha^{-1})	HI (%)	% Grain protein
LEX H	13.9	15.7	76.7	3635.4	2782.0	7.3	70.1	4740.9	62.9	9.8
PRN H	8.3	12.4	85.8	4405.7	2998.4	6.1	70.1	4559.3	60.4	9.7
L	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	0.96	0.03	0.001	0.28
R(L)	0.004	0.00	0.07	0.08	0.01	0.0001	0.16	<0.0001	0.0001	0.81
G	0.001	<0.0001	0.0003	0.58	0.18	0.06	0.97	0.05	0.03	<0.0001
G*L	<0.0001	<0.0001	0.78	0.87	0.57	0.32	0.88	0.37	0.37	0.21

Table 5.4. Yield rankings of genotypes across locations of the most stable lines grown in the 2014 N study Lexington (LEX) and Princeton (PRN), KY under 0 kg N ha^{-1} (L) and 112 kg N ha^{-1} (H) environment.

Genotypes	LEX L	LEX H	PRN L	PRN H
KY93C-1238-17-1	1	10	15	3
KY02C-1121-75	2	5	8	35
KY03C-2314-08	7	2	4	21
IL01-11934	10	6	5	16
KY06C-1003-139-8-3	12	11	9	14
IL07-20743	14	28	1	12

Table 5.5. LSMEANS for N traits in each N environment from the N study Lexington (LEX) and Princeton (PRN), KY 0 (L) and 112 (H) kg N ha^{-1} N environment. Level of significance from ANOVA is shown for each trait. N environment (E), location (L), genotype (G). Post anthesis nitrogen uptake (PANU), nitrogen harvest index (NHI), normalized difference vegetative index (NDVI), nitrogen use efficiency (NUE), nitrogen utilization efficiency (NUtE), nitrogen uptake efficiency (NUpE).

Environment	% N anthesis	% N maturity	N grain (kg ha^{-1})	N veg anthesis (kg ha^{-1})	N veg maturity (kg ha^{-1})	Total plant N (kg ha^{-1})	PANU (kg ha^{-1})	NHI (%)	NDVI	NUE	NUtE (kg kg^{-1})	NUpE
PRNL	1.24	0.36	37.5	47.3	7.4	45.0	-2.4	83.5	0.43	42.1	52.7	0.80
PRN H	1.26	0.28	70.9	55.4	8.2	79.0	23.6	89.7	0.70	29.6	57.7	0.51
LEX L	1.44	0.65	40.6	54.5	14.0	54.8	0.3	74.6	0.46	63.4	48.9	1.30
LEX H	1.80	0.50	74.4	66.4	13.8	88.2	21.8	84.2	0.66	31.1	53.9	0.58
L	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.94	<0.0001	0.3404	<0.0001	<0.0001	<0.0001
R(L)	0.09	<0.0001	0.0041	0.0075	0.0001	<0.0001	0.001	0.02	0.67	0.35	0.10	0.01
E	<0.0001	<0.0001	<0.0001	<0.0001	0.2984	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
G	0.10	0.001	0.15	0.17	0.14	0.45	0.21	0.03	0.01	<0.0001	<0.0001	0.016
G*L	0.15	0.11	0.14	0.87	0.29	0.28	0.48	0.08	0.26	<0.0001	0.110	0.003
G*E	0.57	0.37	0.95	0.69	0.005	0.88	0.89	0.06	0.45	0.22	0.04	0.14
L*E	<0.0001	<0.0001	0.76	0.06	0.08	0.69	0.10	<0.0001	<0.0001	<0.0001	0.93	<0.0001
G*L*E	0.87	0.85	0.98	0.53	0.16	0.94	0.75	0.44	0.82	0.31	0.20	0.32

Table 5.6. Pearson correlation coefficients for agronomic and N traits measured from the 56 soft red winter wheat lines grown in each N environment N study 2014 Lexington, KY location. 0 kg N ha^{-1} treatment correlations depicted at the top of the table. 112 kg N ha^{-1} treatment depicted at the left of the table. Heading date (Hd), anthesis date (Ad), height (H), vegetative biomass at anthesis (Vba), vegetative biomass at maturity (Vbm), Test weight (Twt), yield (Y), harvest index (HI), % grain protein (Gp), % N anthesis in vegetative tissue (Na), % N maturity vegetative tissue (Nm), grain N content (Ngc), vegetative N anthesis content (Nva), vegetative N maturity content (Nma), total plant N content (TN), post-anthesis N uptake (PANU), nitrogen harvest index (NHI), normalized difference vegetative index (NDVI), nitrogen use efficiency (NUE), nitrogen utilization efficiency (NUtE), nitrogen uptake efficiency (NUpE).

	Hd	Ad	H	Vba	Vbm	Twt	Y	HI	Gp	Na	Nm	Ngc	Nva	Nma	TN	PANU	NHI	NDVI	NUE	NUtE	NUpE
Hd	.	0.18	-0.002	0.07	-0.05	-0.02	-0.22	-0.05	0.06	-0.10	0.16	-0.23	0.01	0.10	-0.14	-0.09	-0.19	-0.01	-0.22	-0.17	-0.14
Ad	0.79**	.	0.14	0.05	0.17	0.08	-0.02	-0.19	0.13	-0.04	0.05	0.04	0.01	0.19	0.13	0.07	-0.18	-0.19	-0.02	-0.22	0.13
H	0.26'	0.21	.	-0.09	0.28*	-0.16	0.29*	-0.07	-0.33*'	0.09	-0.34*'	0.19	-0.01	-0.0005	0.15	0.10	0.07	0.19	0.29*	0.21	0.16
Vba	0.04	0.03	-0.03	.	-0.07	-0.03	-0.01	0.05	0.18	0.06	0.05	0.07	0.81**	-0.03	0.03	-0.71**	0.06	-0.30*	-0.01	-0.03	0.03
Vbm	-0.14	-0.05	-0.02	0.25	.	-0.10	0.23	-0.84**	-0.29*	-0.09	-0.38**	0.15	-0.12	0.78**	0.53**	0.43**	-0.64**	0.24	0.23	-0.36**	0.54**
Twt	0.14	0.07	0.12	-0.18	-0.20	.	-0.14	0.06	-0.05	0.14	0.08	-0.18	0.06	-0.07	-0.19	-0.17	-0.004	0.02	-0.14	0.07	-0.19
Y	0.13	-0.05	0.52**	-0.19	-0.12	0.16	.	0.32*	-0.50**	0.35**	-0.14	0.93**	0.21	0.08	0.79**	0.29*	0.35**	0.05	1.00**	0.56**	0.79**
HI	0.19	-0.01	0.40**	-0.27*	-0.70**	0.20	0.79**	.	-0.02	0.29	0.27*	0.35*	0.22	-0.73**	-0.11	-0.27*	0.83**	-0.18	0.32*	0.68**	-0.11
Gp	0.04	0.13	-0.35**	-0.01	0.07	0.08	-0.21	-0.19	.	-0.10	0.29*	-0.14	0.08	-0.06	-0.14	-0.15	0.003	-0.09	-0.50**	-0.60**	-0.14
Na	-0.08	-0.01	-0.07	-0.20	-0.11	0.18	-0.10	-0.03	-0.05	.	0.03	0.35	0.63**	-0.11	0.22	-0.43**	0.27*	-0.02	0.35**	0.29*	0.22
Nm	-0.02	0.11	-0.22	-0.15	-0.14	0.16	-0.02	0.06	0.30	-0.16	.	-0.05	0.06	0.22	0.04	-0.03	-0.24	-0.22	-0.14	-0.30*	0.04
Ngc	0.15	0.01	0.37	-0.19	-0.08	0.20	0.89**	0.69**	0.24	-0.12	0.12	.	0.27*	0.07	0.84**	0.27*	0.40**	0.02	0.93**	0.38**	0.84**
Nva	-0.04	0.00	-0.10	0.43**	0.04	0.05	-0.20	-0.18	-0.03	0.80**	-0.24	-0.23	.	-0.10	0.15	-0.80**	0.21	-0.24	0.21	0.15	0.15
Nma	-0.07	0.09	-0.21	-0.01	0.37**	0.04	-0.07	-0.28*	0.32*	-0.19	0.86**	0.09	-0.19	.	0.58**	0.44**	-0.88**	0.16	0.08	-0.65**	0.58**
TN	0.12	0.05	0.28*	-0.24	-0.01	0.20	0.81**	0.59**	0.32*	-0.14	0.35**	0.96**	-0.27*	0.34*	.	0.47**	-0.13	0.15	0.79**	-0.05	1.00**
PANU	0.11	0.03	0.26'	-0.39*	-0.03	0.13	0.72**	0.54**	0.26'	-0.49**	0.38**	0.84**	-0.68**	0.35**	0.89**	.	-0.27*	0.30*	0.29*	-0.17	0.47**
NHI	0.15	-0.06	0.37**	-0.15	-0.40**	0.05	0.55**	0.65**	-0.12	0.12	-0.66**	0.48**	0.03	-0.81**	0.25	0.18	.	-0.09	0.35**	0.76**	-0.13
NDVI	0.01	-0.11	0.38**	-0.03	0.03	0.22	0.39**	0.27*	0.27*	-0.15	0.07	0.52**	-0.15	0.08	0.50**	0.45**	0.24	.	0.05	-0.09	0.15
NUE	0.13	-0.05	0.52**	-0.18	-0.12	0.16	1.00**	0.79**	-0.21	-0.10	-0.02	0.89**	-0.21	-0.07	0.81**	0.72**	0.55**	0.39**	.	0.57**	0.79**
NUtE	0.05	-0.15	0.45**	-0.05	-0.23	-0.03	0.46**	0.47**	-0.86**	0.11	-0.59**	0.06	0.07	-0.66**	-0.13	-0.13	0.60**	-0.09	0.46**	.	-0.05
NUpE	0.12	0.04	0.28*	-0.24	-0.02	0.20	0.81**	0.59**	0.32*'	-0.15	0.35**	0.96**	-0.28*	0.33*'	1.00**	0.89**	0.26'	0.50**	0.81**	-0.13	.

*p<.05, **p<.01

Table 5.7. Pearson correlation coefficients for agronomic and N traits measured from the 56 soft red winter wheat lines grown in each N environment N study 2014 Princeton, KY location. 0 kg N ha^{-1} treatment correlations depicted above the diagonal. 112 kg N ha^{-1} treatment depicted below the diagonal. Heading date (Hd), anthesis date (Ad), height (H), vegetative biomass at anthesis (Vba), vegetative biomass at maturity (Vbm), Test weight (Twt), yield (Y), harvest index (HI), % grain protein (Gp), % N anthesis in vegetative tissue (Na), % N maturity vegetative tissue (Nm), grain N content (Ngc), vegetative N anthesis content (Nva), vegetative N maturity content (Nma), total plant N content (TN), post-anthesis N uptake (PANU), nitrogen harvest index (NHI), normalized difference vegetative index (NDVI), nitrogen use efficiency (NUE), nitrogen utilization efficiency (NUtE), nitrogen uptake efficiency (NUpE).

	Hd	Ad	H	Vba	Vbm	Twt	Y	HI	Gp	Na	Nm	Ngc	Nva	Nma	TN	PANU	NHI	NDVI	NUE	NUtE	NUpE
Hd	.	0.67**	0.02	-0.06	0.12	0.04	0.01	-0.12	-0.02	0.28	-0.33*	0.01	0.05	-0.23	-0.06	-0.08	0.22	-0.07	0.01	0.12	-0.07
Ad	0.70**	.	0.03	0.04	0.09	-0.04	0.15	0.00	-0.13	0.28*	-0.31*	0.13	0.16	-0.19	0.04	-0.13	0.27*	-0.08	0.15	0.24	0.05
H	-0.21	-0.12	.	0.08	0.25	-0.22	0.48**	0.04	-0.01	-0.08	-0.38**	0.53**	0.05	-0.19	0.44**	0.18	0.43**	0.52**	0.48**	0.25	0.43*
Vba	-0.16	0.05	0.001	.	0.25	-0.07	0.20	-0.11	0.09	-0.35**	-0.21	0.26'	0.92**	-0.0004	0.23	-0.72**	0.12	-0.005	0.20	0.03	0.24
Vbm	-0.06	-0.24	0.08	0.07	.	-0.25	0.28*	-0.81**	-0.005	-0.10	-0.55**	0.30*	0.22	0.34*'	0.39**	0.002	-0.16	-0.11	0.28*	-0.07	0.39**
Twt	-0.03	-0.22	0.21	0.15	0.38**	.	-0.32*	0.02	0.08	0.03	0.21	-0.33*'	-0.06	-0.04	-0.31*	-0.11	-0.11	-0.05	-0.32**	-0.18	-0.31**
Y	-0.10	-0.24	0.33*'	-0.14	0.07	0.30*	.	0.32**	-0.49**	-0.20	-0.30*	0.94**	0.13	-0.08	0.85**	0.33**	0.52**	0.36**	1.00**	0.60**	0.85**
HI	0.03	0.09	0.07	-0.15	-0.84**	-0.16	0.46**	.	-0.27*	-0.04	0.36*	0.27*	-0.13	-0.39**	0.12	0.19	0.48**	0.30*	0.32*	0.42**	0.13
Gp	0.16	0.09	-0.35**	0.02	-0.11	0.08	-0.41**	-0.10	.	-0.17	0.08	-0.16	0.01	0.11	-0.12	-0.08	-0.20	-0.06	-0.49**	-0.75**	-0.11
Na	-0.11	0.09	-0.08	-0.15	0.00	0.09	-0.01	0.02	-0.04	.	-0.06	-0.29*	0.06	-0.10	-0.29*	-0.21	-0.03	-0.09	-0.20	0.08	-0.30*
Nm	0.13	0.26'	-0.27*	0.04	-0.10	-0.18	-0.14	0.02	0.19	0.09	.	-0.29*	-0.24	0.56**	-0.05	0.19	-0.62**	-0.08	-0.30*	-0.51**	-0.05
Ngc	-0.01	-0.21	0.16	-0.15	0.04	0.36**	0.86**	0.43**	0.11	-0.05	-0.07	.	0.15	-0.04	0.92**	0.35*	0.51*	0.39**	0.94**	0.38**	0.93**
Nva	-0.20	0.10	-0.05	0.75**	0.06	0.19	-0.13	-0.12	-0.01	0.53**	0.10	-0.16	.	-0.03	0.12	-0.85**	0.10	-0.04	0.13	0.06	0.13
Nma	0.04	0.09	-0.18	0.05	0.31*	0.04	-0.06	-0.31*	0.15	0.09	0.87**	0.003	0.11	.	0.33*	0.20	-0.86**	-0.21	-0.08	-0.66**	0.32*
TN	0.01	-0.14	0.05	-0.11	0.17	0.33*'	0.72**	0.24	0.16	0.003	0.38	0.87**	-0.09	0.50**	.	0.42**	0.16	0.30*	0.85**	0.10	1.00**
PANU	0.13	-0.16	0.07	-0.55**	0.09	0.13	0.60**	0.25	0.13	-0.33*'	0.21	0.73**	-0.68**	0.30*	0.79**	.	-0.004	0.20	0.33*'	0.001	0.41**
NHI	-0.07	-0.15	0.23	-0.06	-0.25	-0.01	0.19	0.33*'	-0.14	-0.13	-0.88**	0.14	-0.14	-0.97**	-0.36**	-0.18	.	0.37*	0.52**	0.75**	0.17
NDVI	-0.06	-0.06	0.51**	0.01	0.10	0.24	0.44**	0.13	-0.37*	-0.02	-0.17	0.26'	0.001	-0.15	0.16	0.12	0.19	.	0.36**	0.23	0.30*
NUE	-0.10	-0.24	0.32*'	-0.15	0.07	0.30*	1.00**	0.46**	-0.41**	-0.01	-0.14	0.86**	-0.13	-0.06	0.72**	0.60**	0.19	0.44**	.	0.60**	0.85**
NUtE	-0.16	-0.17	0.36**	-0.05	-0.13	-0.02	0.44**	0.33*'	-0.76**	-0.03	-0.68**	0.05	-0.07	-0.73**	-0.31**	-0.19	0.73**	0.39**	0.44**	.	0.10
NUpE	0.003	-0.15	0.07	-0.11	0.15	0.32*	0.72**	0.25	0.16	-0.005	0.36**	0.87**	-0.09	0.48**	1.00**	0.79**	-0.34**	0.17	0.72**	-0.29*	.

*p<.05, **p<.01

Table 5.8. LSMEANS for N traits in each environment from the N study Lexington (LEX) and Princeton (PRN), KY 0 (L) kg N ha^{-1} N environment. Level of significance from ANOVA is shown for each trait. Rep (R), location (L), genotype (G). Post anthesis nitrogen uptake (PANU), nitrogen harvest index (NHI), normalized difference vegetative index (NDVI), nitrogen use efficiency (NUE), nitrogen utilization efficiency (NUtE), nitrogen uptake efficiency (NUpE).

Loc	% N anthesis	% N maturity	N grain (kg ha^{-1})	N veg anthesis (kg ha^{-1})	N veg maturity (kg ha^{-1})	Total plant N (kg ha^{-1})	PANU (kg ha^{-1})	NHI (%)	NDVI	NUE	NUtE (kg kg^{-1})	NUpE
LEX H	1.83	0.50	74.4	66.2	13.8	88.2	21.6	84.2	0.66	31.1	53.9	0.58
PRN H	1.26	0.28	70.9	55.4	8.2	79.0	23.8	89.7	0.70	29.6	57.8	0.51
L	<0.0001	<0.0001	0.02	<0.0001	<0.0001	<0.0001	0.23	<0.0001	<0.0001	0.01	<0.0001	<0.0001
R(L)	0.20	0.0002	<0.0001	0.02	0.01	<0.0001	<0.0001	0.56	0.02	<0.0001	0.73	<0.0001
G	0.37	0.002	0.57	0.48	0.03	0.63	0.08	0.01	0.02	0.05	<0.0001	0.63
G*L	0.92	0.65	0.66	1.00	0.35	0.59	0.58	0.42	0.28	0.36	0.25	0.59

Table 5.9. LSMEANS for N traits each N environment from the N study Lexington (LEX) and Princeton (PRN), KY 112 (H) kg N ha^{-1} environment. Level of significance from ANOVA is shown for each trait. Rep (R), N treatment (E), location (L), genotype (G). Post anthesis nitrogen uptake (PANU), nitrogen harvest index (NHI), normalized difference vegetative index (NDVI), nitrogen use efficiency (NUE), nitrogen utilization efficiency (NUtE), nitrogen uptake efficiency (NUpE).

Loc	% N anthesis	% N maturity	N grain (kg ha^{-1})	N veg anthesis (kg ha^{-1})	N veg maturity (kg ha^{-1})	Total plant N (kg ha^{-1})	PANU (kg ha^{-1})	NHI (%)	NDVI	NUE	NUtE (kg kg^{-1})	NUpE
LEX L	1.44	0.65	40.6	54.5	14.0	54.8	0.3	74.6	0.46	63.4	48.9	1.30
PRN L	1.24	0.36	37.5	47.3	7.4	45.0	-2.4	83.5	0.43	42.1	52.8	0.80
L	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.09	<0.0001	0.004	<0.0001	<0.0001	<0.0001
R(L)	0.014	0.02	0.03	0.30	0.02	0.26	0.04	0.0091	0.33	0.05	0.09	0.36
G	0.27	0.55	0.011	0.13	0.07	0.03	0.46	0.195	0.26	<0.0001	0.02	0.01
G*L	0.16	0.25	0.004	0.02	0.13	0.02	0.04	0.127	0.68	0.0002	0.12	0.01

Table 5.10. Yield, % moisture, and test weight (Twt) LSMEANS for the 56 soft red winter lines grown at Princeton (PRN) and Lexington (LEX), KY under 0 kg N ha^{-1} (L) and 112 kg N ha^{-1} (H). Mean ($\bar{x}$), standard error (SE).

Genotype	Yield (kg ha^{-1})				% Grain Moisture				Twt (kg hL^{-1})			
	PRN		LEX		PRN		LEX		PRN		LEX	
	L	H	L	H	L	H	L	H	L	H	L	H
011007A1-14-16-50	2047.7	4382.2	2053.3	4025.2	22.4	21.8	14.3	14.4	71.1	69.0	68.4	70.9
03207A1-7-3-1	2102.6	4035.7	2215.9	3969.3	20.9	23.5	14.4	14.6	69.9	70.3	70.0	70.8
03633A1-69-2-5	2298.4	4238.9	2048.4	4080.7	21.6	20.2	14.7	14.7	70.5	69.3	70.9	68.8
04620A1-1-7-4	2397.4	4665.5	2234.1	4256.6	20.8	21.3	14.5	14.3	68.3	69.3	70.3	69.4
04719A1-16-1-1-7	2277.4	4292.3	2088.2	3920.4	21.6	20.6	14.1	14.2	70.2	68.6	69.6	69.3
05219A1-8-21-2-4	2289.0	4406.1	2020.1	4686.6	20.8	21.3	14.3	14.0	70.1	69.4	67.4	70.5
05222A1-1-2-1	2187.6	4527.2	2233.4	4167.0	21.9	21.4	14.2	14.2	70.0	69.6	66.9	70.5
0537A1-3-12	2336.6	5142.5	2739.6	4849.8	20.9	20.4	14.5	14.6	70.1	69.8	68.1	71.0
07290A1-12	2066.7	4620.3	2569.2	4075.5	22.4	22.7	14.5	14.2	70.2	70.1	69.4	70.4
ALLEGIANCE	2210.9	4165.1	2636.1	4922.3	22.0	21.5	14.6	14.4	67.9	69.3	69.0	70.0
FOSTER	2284.7	4535.2	2753.9	4798.9	19.7	22.4	14.5	14.0	69.3	70.0	69.6	71.1
IL01-11934	2748.2	4762.9	3035.6	5567.2	22.3	22.9	14.6	14.6	69.9	72.6	67.1	69.2
IL06-13072	2571.1	4990.9	2446.6	4963.3	23.2	22.2	14.5	14.5	69.8	71.5	67.1	70.6
IL06-7550	2392.4	5069.4	2614.5	5132.3	21.9	22.8	14.4	14.3	70.0	69.6	70.1	71.6
IL07-19334	2388.7	5075.0	2570.5	3905.1	22.2	24.0	14.6	14.4	69.3	69.0	68.0	69.7
IL07-20728	2210.8	4931.2	2542.4	6227.8	20.9	23.0	14.4	14.2	69.6	71.2	68.7	69.9
IL07-20743	2886.4	4826.6	2909.6	4702.8	22.3	22.0	14.4	14.3	70.2	69.1	68.6	70.0
IL07-21847	2469.5	4263.8	2861.2	4845.7	21.3	22.3	14.4	14.3	70.2	70.2	68.3	69.2
IL07-23420	2157.9	4673.9	3117.5	5142.5	21.8	22.3	14.6	13.9	70.0	71.0	70.1	71.2
IL07-6861	2846.8	4624.1	2636.9	4618.4	21.2	22.3	14.3	14.3	70.9	69.7	71.4	70.6
IL08-34020	2628.4	4698.9	2118.0	3447.2	21.6	21.8	14.4	14.5	69.6	70.9	67.4	71.6
IL99-26442	2352.5	4180.4	3138.2	4582.8	22.4	23.0	14.7	14.4	69.5	69.1	68.6	71.3
KY02C-1058-03	2621.0	4252.3	2359.4	4394.0	21.1	21.7	14.9	14.4	70.0	69.7	70.6	69.0
KY02C-1076-07	2316.0	4572.4	2805.9	4620.0	22.0	20.9	14.7	14.6	70.2	70.5	69.4	69.1
KY02C-1121-11	1802.0	4193.6	3067.6	5701.0	20.7	21.6	14.6	14.3	71.5	70.7	69.8	71.4
KY02C-1121-75	2663.5	4448.0	3327.4	5665.2	21.6	21.9	14.8	14.4	68.3	70.3	69.3	70.2
KY02C-1122-06	2194.7	4834.1	2399.3	4522.4	21.0	22.0	14.5	14.3	70.6	70.5	70.1	70.3
KY02C-2215-02	2436.1	4933.9	2750.1	5080.9	22.1	22.7	14.6	14.6	69.6	71.7	67.3	68.8
KY02C-3004-07	2223.0	4172.6	2726.7	5426.9	22.1	21.9	14.8	14.4	69.0	69.0	69.6	68.5
KY02C-3005-25	2754.8	4688.5	2760.3	4391.7	21.5	22.6	14.5	14.4	69.8	70.5	69.3	68.5
KY03C-1002-02	2726.4	5201.0	2609.9	5142.3	20.7	22.2	14.5	14.1	68.6	70.5	71.2	70.1
KY03C-1192-37	2677.4	4738.4	2776.1	4456.1	21.1	22.4	14.6	14.5	68.8	70.8	70.3	72.4
KY03C-1195-10-1-5	2278.6	4370.1	2889.6	4552.3	21.2	23.1	14.8	14.8	69.3	70.5	68.4	68.6
KY03C-1221-01	2261.9	4339.9	2103.3	3804.3	21.7	21.7	14.7	14.3	67.7	71.7	71.9	69.0
KY03C-1221-06	2219.1	4221.2	2685.7	3819.3	22.1	22.7	14.7	14.8	71.2	69.4	72.6	70.3
KY03C-1221-22	2302.4	4480.7	2186.4	4058.2	22.0	21.7	14.5	14.1	70.2	71.3	70.2	69.6
KY03C-1237-01	2437.4	4477.8	2947.2	5804.7	21.9	21.1	14.7	14.3	67.7	70.0	68.6	71.5
KY03C-1237-15	2642.5	4229.1	2481.4	4643.5	21.1	21.4	14.2	14.4	68.3	67.3	70.3	71.1
KY03C-1237-32	2573.4	4371.6	2674.9	4429.7	20.8	21.7	14.6	14.3	70.2	69.7	68.5	66.9
KY03C-2047-02	2465.9	4662.8	2614.2	4751.1	20.7	22.9	14.6	14.7	70.0	70.2	70.0	69.9
KY03C-2047-06	2375.2	4818.3	2759.7	4549.0	22.2	20.7	14.5	14.5	71.3	70.9	70.1	69.3
KY03C-2049-02	1987.2	4003.8	3162.0	4111.0	22.3	21.2	14.5	14.2	70.8	69.0	68.5	69.6
KY03C-2314-08	2748.3	4685.3	3073.6	6174.0	20.8	23.0	14.8	14.4	70.4	70.8	69.4	70.0
KY03C-2399-02	1848.0	4863.2	2564.6	4272.0	22.8	21.2	14.4	14.3	71.8	71.0	71.3	71.3
KY04C-1128-38-1-5	2465.6	4605.1	2804.7	4111.0	22.1	21.3	14.7	14.5	69.5	71.0	68.2	69.8
KY04C-2006-41-1-1	2217.4	4957.6	3260.8	5053.4	21.9	22.4	14.5	14.5	70.0	70.4	69.2	69.7
KY04C-2151-40	2372.6	4547.7	2520.2	4807.4	22.2	20.3	14.3	14.1	69.8	71.6	69.0	70.8
KY04C-2151-41	2332.0	4208.2	2431.6	4751.5	21.2	21.5	14.4	14.3	68.7	69.6	69.7	67.6
KY04C-3006-33-14-3	2145.5	3781.6	2858.7	5125.4	21.1	21.8	14.3	14.6	70.7	69.9	65.8	70.1
KY05C-1007-2-12-5	2426.1	4797.1	2875.4	5536.5	20.3	21.7	14.7	14.4	69.3	70.1	68.3	70.4
KY05C-1105-42-20-1	2153.7	4211.9	2496.5	5510.7	20.9	20.3	14.6	14.7	68.7	71.0	71.2	71.0
KY05C-1381-77-7-5	2420.3	4278.0	2765.8	5094.1	21.4	22.5	14.7	14.6	69.9	69.3	69.0	71.2
KY05C-1617-17-17-3	2434.3	4646.5	3023.4	4519.8	21.8	21.4	14.7	14.5	68.8	71.9	71.8	68.2
KY06C-1003-139-8-3	2658.9	4814.0	2955.3	5229.7	20.9	21.8	14.9	14.6	68.9	69.0	68.6	69.8
KY93C-1238-17-1	2478.2	5115.2	3375.5	5349.7	21.8	23.0	14.6	14.5	68.6	69.3	68.2	73.0
PEMBROKE	1993.5	4692.9	3053.6	5144.5	20.4	22.0	14.9	14.5	70.4	70.1	69.1	72.5
$\bar{x}$	2371.5	4559.3	2673.7	4740.9	21.5	21.9	14.5	14.4	69.7	70.1	69.3	70.1
SE	32.2	43.0	46.1	82.3	0.09	0.11	0.02	0.03	0.12	0.13	0.18	0.16

Table 5.11. N in vegetative content at maturity (Nvm), Grain N content (Ngc), Total plant N content (TN), and nitrogen harvest index (NHI) LSMEANS for 56 soft red winter lines grown at Princeton (PRN) and Lexington (LEX), KY under 0 kg N ha^{-1} (L) and 112 kg N ha^{-1} (H). Mean ($\bar{x}$), standard error (SE).

Genotype	Nvm (kg ha^{-1})				Ngc (kg ha^{-1})				TN (kg ha^{-1})				NHI (%)			
	PRN		LEX		PRN		LEX		PRN		LEX		PRN		LEX	
	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
011007A1-14-16-50	5.9	8.3	12.5	19.8	33.6	70.9	32.8	67.1	39.5	79.3	45.4	86.9	85.1	89.5	72.2	77.3
03207A1-7-3-1	7.8	10.4	16.0	12.7	34.0	68.0	36.9	75.5	41.7	78.4	52.9	88.2	81.4	86.6	70.0	84.6
03633A1-69-2-5	6.2	16.4	9.7	15.4	36.9	68.7	34.0	58.3	43.1	85.1	43.7	73.8	86.0	80.4	77.9	78.7
04620A1-1-7-4	7.7	7.7	16.9	12.6	39.7	70.7	35.9	66.9	47.5	78.5	55.7	79.5	83.8	90.2	69.6	84.3
04719A1-16-1-1-7	8.2	8.1	10.7	13.6	37.9	68.3	33.3	65.9	46.1	76.4	44.1	79.4	82.1	89.5	75.6	82.7
05219A1-8-21-2-4	7.2	7.8	13.7	18.8	36.7	73.7	33.3	83.9	44.0	81.5	49.0	102.7	83.5	90.4	72.3	81.6
05222A1-1-2-1	8.3	12.8	15.0	17.0	35.4	70.2	36.3	70.7	43.7	83.0	51.9	87.6	81.0	84.6	71.2	80.4
0537A1-3-12	6.9	9.7	14.6	10.5	38.6	79.9	41.2	78.4	45.5	89.6	55.8	94.5	84.8	89.2	73.9	89.2
07290A1-12	6.7	7.0	12.8	13.2	32.2	72.2	39.2	71.1	38.9	79.2	51.9	84.3	82.7	91.2	75.4	84.0
ALLEGIANCE	5.7	7.9	17.7	12.2	33.6	67.6	38.8	74.9	39.3	75.5	56.6	87.2	84.4	89.7	68.8	86.0
FOSTER	6.6	9.9	13.0	13.2	36.4	69.1	41.0	82.8	43.0	79.0	54.0	95.9	84.7	87.4	76.1	86.3
IL01-11934	5.2	6.9	10.9	15.6	42.2	83.5	43.5	82.5	47.4	90.4	57.2	98.2	89.2	92.3	81.0	84.1
IL06-13072	6.6	6.8	12.6	12.2	39.9	74.8	37.5	73.0	46.5	81.5	50.0	85.2	85.5	92.1	75.2	85.5
IL06-7550	5.7	7.5	14.5	14.1	38.9	71.9	36.8	78.6	44.6	79.3	51.3	92.7	87.6	90.7	72.0	84.8
IL07-19334	6.1	7.5	16.7	15.3	36.8	75.1	37.0	54.1	42.9	82.7	53.7	69.4	85.7	90.9	68.9	77.8
IL07-20728	6.6	7.0	12.8	13.4	34.5	76.4	38.2	94.3	41.1	83.4	51.0	107.7	83.2	91.6	75.2	87.2
IL07-20743	7.3	5.8	12.5	13.4	44.9	76.0	43.6	70.8	55.3	81.8	56.1	75.8	86.8	93.3	77.8	82.5
IL07-21847	7.7	11.4	15.6	13.4	38.6	65.5	41.3	71.9	46.3	76.8	56.9	85.3	83.6	84.6	72.7	84.4
IL07-23420	6.3	8.8	16.3	15.0	34.3	71.4	46.0	83.1	40.6	80.2	62.4	98.1	84.3	88.9	73.9	85.0
IL07-6861	5.4	9.2	10.1	19.7	43.1	71.3	39.9	80.2	48.5	80.5	50.0	99.9	89.5	88.4	79.8	79.5
IL08-34020	5.5	9.9	13.5	14.8	39.7	71.3	30.7	52.0	45.3	81.2	44.2	66.9	87.9	87.9	69.5	78.0
IL99-26442	8.6	6.5	11.6	12.4	38.3	63.5	47.8	71.8	46.9	70.0	59.4	84.3	81.7	90.8	80.7	84.9
KY02C-1058-03	7.1	7.0	16.8	15.2	40.2	66.5	38.3	72.5	47.3	73.5	55.1	87.8	85.0	90.6	69.5	82.4
KY02C-1076-07	8.1	8.1	12.7	13.9	36.3	68.6	40.9	70.6	44.4	76.7	53.6	84.5	81.8	89.5	76.4	83.4
KY02C-1121-11	9.5	10.0	11.4	19.1	29.2	65.7	44.8	92.8	38.7	75.7	56.2	111.9	76.2	86.7	80.0	82.2
KY02C-1121-75	8.4	6.7	16.5	10.8	41.3	65.6	47.1	83.4	49.8	72.4	63.6	94.2	83.1	91.1	74.2	88.5
KY02C-1122-06	7.6	0.9	7.4	13.3	34.6	73.9	35.8	70.1	42.3	74.8	43.2	83.4	81.9	98.8	83.0	84.0
KY02C-2215-02	6.3	8.5	13.0	11.4	38.4	74.9	44.5	78.2	44.6	83.4	57.5	89.7	85.8	89.7	77.9	87.3
KY02C-3004-07	7.3	7.9	16.6	19.0	34.4	64.6	44.6	89.4	41.7	72.5	61.2	108.4	82.6	89.4	72.8	82.7
KY02C-3005-25	7.2	7.4	8.8	13.4	42.2	72.1	41.1	68.6	49.3	79.5	50.0	82.0	85.6	90.7	81.9	83.9
KY03C-1002-02	8.1	5.6	13.7	15.9	44.1	81.2	39.4	86.8	52.1	86.9	53.1	102.7	84.4	93.4	73.7	84.1
KY03C-1192-37	5.3	10.7	11.7	15.7	43.5	72.5	41.8	67.5	48.7	83.2	53.5	83.2	89.4	87.1	78.1	81.1
KY03C-1195-10-1-5	7.7	4.4	14.7	10.0	35.5	69.9	44.2	69.8	43.2	73.0	59.0	79.8	82.2	93.9	75.0	87.5
KY03C-1221-01	6.8	12.0	9.1	15.8	37.2	68.8	33.2	66.1	44.0	80.8	44.5	81.9	84.5	85.1	79.8	80.6
KY03C-1221-06	7.9	10.3	17.5	16.0	36.1	68.8	41.2	61.4	44.0	79.1	58.7	77.4	82.0	86.8	70.2	79.2
KY03C-1221-22	7.4	7.5	16.0	17.2	35.5	70.1	32.7	60.7	42.9	77.7	48.7	78.0	82.8	90.3	67.1	77.9
KY03C-1237-01	6.1	8.3	15.3	16.3	36.8	69.5	47.5	94.0	42.9	77.7	62.8	110.3	85.8	89.3	75.6	85.8
KY03C-1237-15	10.2	6.7	21.3	16.5	41.1	64.1	36.8	69.0	51.3	70.8	58.0	85.5	80.1	90.6	62.9	81.0
KY03C-1237-32	8.9	4.4	15.8	11.4	41.4	66.1	43.6	70.0	50.3	70.4	59.4	81.4	82.5	94.0	73.8	86.0
KY03C-2047-02	9.1	11.6	11.6	12.7	38.7	74.2	38.2	73.8	47.8	85.8	49.8	86.4	80.9	86.5	76.6	85.3
KY03C-2047-06	7.2	11.0	14.2	9.3	37.2	74.5	41.6	67.8	44.5	85.5	55.8	77.1	83.6	87.1	74.8	87.9
KY03C-2049-02	9.9	5.0	11.5	14.6	32.5	64.5	48.2	63.5	42.4	67.9	59.7	78.2	76.7	95.2	80.8	81.0
KY03C-2314-08	8.6	7.3	10.6	14.7	40.4	69.3	43.5	93.0	49.0	76.6	54.1	107.7	82.5	90.4	80.4	85.9
KY03C-2399-02	6.7	4.3	19.5	9.9	29.8	73.2	38.5	62.6	36.5	77.5	59.6	72.6	81.5	94.4	67.4	86.3
KY04C-1128-38-1-5	5.5	10.6	16.2	9.2	39.0	71.8	42.3	61.7	44.5	82.4	60.1	70.9	87.7	87.6	73.2	87.0
KY04C-2006-41-1-1	7.9	10.0	14.8	11.2	34.5	77.4	47.0	74.4	42.4	87.5	61.8	85.6	81.4	88.5	76.2	87.0
KY04C-2151-40	7.5	8.5	15.5	13.7	39.8	72.9	40.9	80.5	47.3	81.4	56.4	94.2	84.0	89.8	72.2	85.0
KY04C-2151-41	9.5	8.6	13.4	16.6	40.6	67.4	38.8	76.6	50.1	76.0	52.2	93.2	81.0	89.2	74.3	81.8
KY04C-3006-33-14-3	5.1	5.4	18.2	11.4	35.5	61.2	46.5	79.8	40.6	66.5	64.7	91.3	87.5	92.3	71.8	87.4
KY05C-1007-2-12-5	8.8	5.3	13.6	12.3	38.2	72.6	42.9	82.9	47.0	78.0	56.4	95.2	81.5	93.2	76.0	87.1
KY05C-1105-42-20-1	8.3	9.7	9.9	11.0	36.6	65.1	38.1	84.3	44.9	74.7	48.0	95.4	81.5	87.1	79.3	88.2
KY05C-1381-77-7-5	8.1	7.4	16.0	8.1	36.7	68.3	39.2	83.5	44.7	75.7	55.2	91.5	82.0	90.3	70.8	91.1
KY05C-1617-17-17-3	9.9	9.5	15.5	9.3	36.8	70.7	44.7	68.4	46.7	80.1	60.2	77.7	79.7	88.3	74.2	87.7
KY06C-1003-139-8-3	10.5	8.1	18.6	14.4	39.9	76.0	41.2	77.7	50.4	84.1	59.8	92.1	79.7	90.7	68.8	84.2
KY93C-1238-17-1	6.1	12.7	15.4	14.2	37.6	76.1	48.7	78.7	43.7	88.9	64.2	92.9	85.9	85.9	76.1	84.8
PEMBROKE	8.6	6.4	9.8	8.6	33.3	70.5	50.3	81.0	41.9	76.9	60.1	89.6	79.4	91.8	83.7	90.4
$\bar{x}$	7.4	8.2	14.0	13.8	37.5	70.9	40.6	74.4	45.0	79.0	54.8	88.2	83.5	89.7	74.6	84.2
SE	0.18	0.34	0.39	0.38	0.45	0.60	0.62	1.30	0.50	0.70	0.74	1.41	0.38	0.40	0.59	0.44

Table 5.12. Association mapping of measured traits and markers using the Mixed Linear Model in TASSEL from the 2014 Lexington, KY 0 kg N ha^{-1} environment. Bonferroni test correction was used to identify significant markers. Anthesis date (Ad), heading date (hd), % grain protein (Gp), N use efficiency (NUE), N uptake efficiency (NUpE), total plant N (TN), yield (Y).

Trait	Marker	Locus	Site	F	p	Marker R^2
Ad	GENE-4204_738	6B	7342	106.1	2.42E-18**	0.12
Ad	Kukri_c55163_274	6B	7342	106.1	2.42E-18**	0.12
Ad	Excalibur_rep_c69189_235	6B	7342	32.1	1.4E-09**	7.2
Ad	GENE-4086_659	6B	7342	32.1	1.4E-09**	7.2
Ad	Kukri_c59960_211	6B	7342	32.1	1.4E-09**	7.2
Ad	RAC875_c22539_859	6B	7342	32.1	1.4E-09**	7.2
Ad	RAC875_c31274_196	6B	7342	32.1	1.4E-09**	7.2
Ad	IAAV4883	6B	6604	31.5	1.6E-09**	1.24
Ad	RFL_Contig897_207	---	---	30.2	3.3E-09**	30.2
Ad	Kukri_c14596_265	---	---	27.2	1.13E-08**	1.27
Ad	IWA168	---	---	21.5	1.94E-07**	0.90
Ad	IWA7506	6B	7264	18.2	1.28E-06**	0.40
Ad	Kukri_c48283_78	6B	12225	15.6	5.77E-06	0.63
Ad	Excalibur_c13714_925	6B	12226	15.6	5.77E-06	0.63
Ad	RAC875_rep_c104893_620	6B	12292	15.6	5.77E-06	0.63
Ad	Tdurum_contig86933_317	4B	7091	8.2	8.85E-04	0.32
Hd	Kukri_c7622_912	---	---	22.7	1.2E-07*	47.5
Hd	IWA5923	5A	1586	13.6	5.63E-04	0.27
Hd	BS00000006_51	5A	14726	12.6	8.41E-04	0.25
Hd	BS00011915_51	---	---	12.4	9.31E-04	0.24
Hd	BS00021860_51	5A	14175	12.4	9.31E-04	0.24
Hd	BS00069245_51	5A	14175	12.4	9.31E-04	0.24
Hd	IACX5640	5A	14175	12.4	9.31E-04	0.24
NUE	IWA1799	2Dx	303	13.3	6.39E-04	0.26
NUpE	RAC875_c5802_144	4B	11545	14.6	3.75E-04	0.28
NUtE	BS00011630_51	---	---	12.4	9.51E-04	0.25
Gp	BS00066209_51	7D	18887	13.9	4.98E-04	0.26
Gp	Tdurum_contig64910_298	---	---	12.9	7.46E-04	0.24
TN	RAC875_c5802_144	4B	11545	14.6	3.75E-04	0.28
Y	IWA1799	2Dx	303	13.3	6.39E-04	0.26

**$p<4.7*10^{-7}$; *$p<2.4*10^{-6}$

Table 5.13. Association mapping of measured traits and markers using the Mixed Linear Model in TASSEL from the 2014 Lexington, KY 112 kg N ha^{-1} environment. Bonferroni test correction was used to identify significant markers. Vegetative biomass maturity (Vbm), N grain content (Ngc), heading date (hd), height (H), vegetative N anthesis (Na), N uptake efficiency (NUpE), grain protein (Gp), total plant N (TN), test weight (Twt).

Trait	Marker	Locus	Site	F	p	Marker R^2
Vbm	Tdurum_contig54785_216	5A	8177	15.3	2.75E-04	0.29
Vbm	IAAV1120	4B	1202	15.2	2.94E-04	0.29
Ngc	Ku_c34010_1016	2B	9311	13.4	6.18E-04	0.26
Ngc	BS00075635_51	---	---	12.5	9.25E-04	0.25
Ngc	BS00068446_51	5A	1569	12.5	9.25E-04	0.25
Hd	Tdurum_contig59631_198	---	---	9.6	3.13E-04	0.37
Hd	Tdurum_contig59965_403	---	---	9.6	3.13E-04	0.37
Hd	BS00021805_51	5A	6764	8.8	5.55E-04	0.35
H	Tdurum_contig42522_573	---	---	16.1	2.04E-04	0.31
H	BS00063578_51	---	---	13.1	6.93E-04	0.26
Na	BS00099805_51	7A	11843	15.6	2.43E-04	0.30
NUpE	BS00066128_51	7D	14586	12.8	8.09E-04	0.26
Gp	Excalibur_c62042_175	3A	2415	15.9	2.31E-04	0.29
Gp	BobWhite_rep_c66748_215	6B	4802	8.6	6.51E-04	0.30
Gp	BobWhite_rep_c66748_275	6B	4802	8.6	6.51E-04	0.30
Gp	IWA4745	---	---	8.3	7.99E-04	0.30
Gp	RAC875_c29042_1124	6B	4802	8.3	7.99E-04	0.30
TN	BS00066128_51	7D	14586	12.8	8.09E-04	0.26
Twt	Tdurum_contig21737_203	---	---	15.3	2.83E-04	0.29
Twt	BobWhite_c16635_331	---	---	12.6	8.67E-04	0.23
Twt	IWA206	---	---	8.1	9.05E-04	0.30

**$p<4.7*10^{-7}$; *$p<2.4*10^{-6}$

Table 5.14. Association mapping of measured traits and markers using the Mixed Linear Model in TASSEL from the 2014 Princeton, KY 0 kg N ha^{-1} environment. Bonferroni test correction was used to identify significant markers. Height (H), N uptake efficiency (NUpE), total plant N (TN), test weight (Twt), vegetative biomass maturity (Vbm).

Trait	Marker	Locus	Site	F	p	Marker R^2
H	Tdurum_contig25539_248	2A	15130	12.8	8.44E-04	0.26
NUpE	BobWhite_c47953_126	---	---	15.1	3.05E-04	0.29
NUpE	RFL_Contig5374_583	---	---	14.6	3.72E-04	0.28
NUpE	BS00063251_51	2Dx	675	13.6	5.62E-04	0.28
NUpE	Excalibur_c94336_103	2Dx	675	13.6	5.62E-04	0.28
NUpE	Excalibur_c94336_68	2Dx	675	13.6	5.62E-04	0.28
NUpE	RFL_Contig5625_912	2Dx	675	13.6	5.62E-04	0.28
NUpE	BS00022224_51	---	---	8.7	5.84E-04	0.34
NUpE	BS00022957_51	---	---	8.7	5.84E-04	0.34
NUpE	BS00049370_51	---	---	8.7	5.84E-04	0.34
NUpE	BS00095512_51	---	---	8.7	5.84E-04	0.34
NUpE	Tdurum_contig77522_310	2A	2597	8.7	5.84E-04	0.34
NUpE	Excalibur_c60081_474	2Dx	675	8.7	5.84E-04	0.34
NUpE	IAAV7407	2Dx	675	8.7	5.84E-04	0.34
NUpE	BS00063632_51	---	---	8.7	5.96E-04	0.34
NUpE	GENE-1343_886	---	---	8.1	8.81E-04	0.32
NUpE	IACX8451	---	---	8.1	8.81E-04	0.32
TN	BobWhite_c47953_126	---	---	15.1	3.05E-04	0.29
TN	RFL_Contig5374_583	---	---	14.6	3.72E-04	0.28
TN	BS00063251_51	2Dx	675	13.6	5.62E-04	0.28
TN	Excalibur_c94336_103	2Dx	675	13.6	5.62E-04	0.28
TN	Excalibur_c94336_68	2Dx	675	13.6	5.62E-04	0.28
TN	RFL_Contig5625_912	2Dx	675	13.6	5.62E-04	0.28
TN	BS00022224_51	---	---	8.7	5.84E-04	0.34
TN	BS00022957_51	---	---	8.7	5.84E-04	0.34
TN	BS00049370_51	---	---	8.7	5.84E-04	0.34
TN	BS00095512_51	---	---	8.7	5.84E-04	0.34
TN	Tdurum_contig77522_310	2A	2597	8.7	5.84E-04	0.34
TN	Excalibur_c60081_474	2Dx	675	8.7	5.84E-04	0.34
TN	IAAV7407	2Dx	675	8.7	5.84E-04	0.34
TN	BS00063632_51	---	---	8.7	5.96E-04	0.34
TN	GENE-1343_886	---	---	8.1	8.81E-04	0.32
TN	IACX8451	---	---	8.1	8.81E-04	0.32
Twt	Excalibur_c40617_983	2A	11601	8.3	8.00E-04	0.32
Vbm	BobWhite_rep_c63085_120	3B	6072	9.4	3.60E-04	0.35
Vbm	BobWhite_c47953_126	---	---	14.3	4.13E-04	0.27
Vbm	IWA280	---	---	8.9	5.10E-04	0.33
Vbm	CAP8_c9110_427	4D	8068	8.6	6.45E-04	0.32
Vbm	Tdurum_contig42418_1811	6A	13685	8.3	8.01E-04	0.31
Vbm	Excalibur_c15246_295	---	---	8.2	8.49E-04	0.32
Vbm	RAC875_c63814_251	3B	6745	8.2	8.49E-04	0.32
Vbm	BS00033372_51	---	---	8.2	8.60E-04	0.31
Vbm	Excalibur_c100910_239	2B	6620	8.1	9.28E-04	0.30
Vbm	IWA2440	2B	6620	8.1	9.28E-04	0.30
Vbm	IWA7120	2B	6620	8.1	9.28E-04	0.30
Vbm	IWA7799	2B	6620	8.1	9.28E-04	0.30
Vbm	RFL_Contig5337_1453	5B	16132	8.1	9.51E-04	0.32

**$p<4.7*10^{-7}$; *$p<2.4*10^{-6}$

Table 5.15. Association mapping of measured traits and markers using the Mixed Linear Model in TASSEL from the 2014 Princeton, KY 112 kg N ha^{-1} environment. Bonferroni test correction was used to identify significant markers. Height (H), N grain content (Ngc), % vegetative N anthesis, N harvest index, % vegetative maturity, N uptake efficiency, % grain protein (Gp), test weight (Twt)

Trait	Marker	Locus	Site	F	p	Marker R^2
Ngc	BobWhite_c27438_81	1A	9446	9.3	3.84E-04	0.35
Ngc	IACX11794	7D	2590	9.0	4.72E-04	0.33
Ngc	Ra_c29886_494	---	---	9.0	4.76E-04	0.34
Ngc	IACX6214	3B	3373	8.6	6.32E-04	0.32
Ngc	RAC875_c60218_63	4D	8384	8.3	7.80E-04	0.31
Ngc	RFL_Contig1323_544	7D	2285	8.3	7.86E-04	0.31
Na	Excalibur_rep_c94717_2115	3B	6745	9.8	2.60E-04	0.36
Na	RAC875_c3956_275	3B	1156	9.6	2.96E-04	0.35
Na	IAAV213	1A	7614	9.6	2.98E-04	0.35
Na	IWA2995	1A	7614	9.6	2.98E-04	0.35
Na	RAC875_c41275_131	1A	7614	9.6	2.98E-04	0.35
Na	Ra_c58315_265	1A	7614	9.6	2.98E-04	0.35
Na	RAC875_c26469_480	2B	7670	9.6	3.12E-04	0.35
Na	RAC875_c15229_108	---	---	9.5	3.21E-04	0.35
Na	RAC875_c3910_1425	---	---	9.5	3.25E-04	0.35
Na	IWA2999	3B	6745	9.4	3.48E-04	0.35
NHI	BS00032406_51	---	---	13.9	4.85E-04	0.27
NHI	BobWhite_c1295_470	---	---	13.9	4.85E-04	0.27
NHI	Tdurum_contig10595_633	---	---	13.9	4.85E-04	0.27
NHI	Tdurum_contig28121_180	---	---	13.9	4.85E-04	0.27
NHI	Tdurum_contig29054_113	---	---	13.9	4.85E-04	0.27
NHI	Tdurum_contig29054_478	---	---	13.9	4.85E-04	0.27
NHI	GENE-4587_50	7A	12290	13.9	4.85E-04	0.27
NHI	IWA6331	7A	12290	13.9	4.85E-04	0.27
NHI	Kukri_c15594_386	7A	12290	13.9	4.85E-04	0.27
NHI	Kukri_c63336_279	7A	12290	13.9	4.85E-04	0.27
NHI	RAC875_c7988_1588	7A	12290	13.9	4.85E-04	0.27
NHI	RAC875_c101928_381	7A	12290	13.6	5.71E-04	0.27
NHI	RAC875_c202_474	4B	9556	12.5	8.90E-04	0.25
NHI	RAC875_c34650_378	---	---	12.3	9.88E-04	0.24
Nm	GENE-4142_88	---	---	12.8	8.03E-04	0.24
Nm	GENE-4142_882	---	---	12.8	8.03E-04	0.24
NUpE	BobWhite_c34068_833	7B	13359	14.1	4.66E-04	0.27
NUpE	Excalibur_c3698_739	7B	13359	12.7	8.44E-04	0.25
Gp	IAAV9104	7D	2285	12.9	3.23E-05	0.50
Gp	TA002853-0110-w	6D	1701	11.6	7.90E-05	0.47
Gp	Kukri_c55362_75	6D	1900	11.6	7.90E-05	0.47
Gp	CAP7_c1404_72	---	---	11.4	8.87E-05	0.44
Gp	IACX1055	---	---	11.4	8.87E-05	0.44
Gp	RAC875_c103967_76	---	---	11.4	8.87E-05	0.44
Gp	RAC875_c61597_406	---	---	11.4	8.87E-05	0.44
Gp	RFL_Contig3135_689	---	---	11.4	8.87E-05	0.44
Gp	RFL_Contig4162_1285	---	---	11.4	8.87E-05	0.44
Gp	Excalibur_c11242_301	---	---	11.4	9.24E-05	0.48
Gp	IAAV1383	4A	11271	11.2	9.56E-05	0.44
Gp	IWA1896	6D	1701	11.3	9.82E-05	0.46
Twt	GENE-0293_65	7B	5705	13.8	5.29E-04	0.27
Twt	BS00003726_51	7B	5863	13.8	5.29E-04	0.27
Twt	BobWhite_c10448_80	7D	11529	13.8	5.29E-04	0.27
Twt	Excalibur_c22903_710	7B	5844	13.4	6.27E-04	0.26
Twt	IWA2832	7B	5375	13.0	7.47E-04	0.26
Twt	IWA5565	7B	5400	13.0	7.47E-04	0.26

**$p<4.7*10^{-7}$; *$p<2.4*10^{-6}$

Table 5.16. Broad sense heritability (h^2) and 90% confidence interval (upper limit (UL), and lower limit (LL)) for N traits across N environments and locations calculated from means squares from ANOVA. N grain content (Ngc), % N anthesis (Na), % N maturity (Nm), normalized difference vegetative index (NDVI), vegetative N content maturity (Vnm), vegetative N content anthesis (Vna), total plant N (TN), post-anthesis N uptake (PANU), nitrogen harvest index (NHI), N utilization efficiency (NUtE), N uptake efficiency (NUpE), N use efficiency (NUE)

Trait	h2	LL	UL
Ngc	0.51	0.30	0.65
Na	0.38	0.17	0.58
Nm	0.57	0.39	0.70
NDVI	0.51	0.28	0.64
Vnm	0.10	-0.38	0.31
Vna	0.25	0.12	0.43
TN	0.32	0.04	0.52
PANU	0.28	-0.01	0.49
NHI	0.31	0.03	0.51
NUtE	0.58	0.41	0.71
NUpE	0.29	0.01	0.50
NUE	0.64	0.50	0.75

Table 5.17. N use efficiency (NUE), N utilization efficiency (NUtE), and N uptake efficiency (NUpE) LSMEANS for 56 soft red winter lines grown at Princeton (PRN) and Lexington (LEX), KY under 0 kg N ha^{-1} (L) and 112 kg N ha^{-1} (H). Mean ($\bar{x}$), standard error (SE).

Genotype	NUE				NUtE ($kg_{yield}\ kg^{-1}_{plant\ N}$)				NUpE			
	PRN		LEX		PRN		LEX		PRN		LEX	
	L	H	L	H	L	H	L	H	L	H	L	H
011007A1-14-16-50	36.4	28.4	48.7	26.4	51.8	55.3	45.1	46.4	0.70	0.51	1.08	0.57
03207A1-7-3-1	37.3	26.2	52.5	26.0	50.4	51.5	43.0	44.9	0.74	0.51	1.25	0.58
03633A1-69-2-5	40.8	27.5	48.6	26.7	53.3	49.8	46.7	53.6	0.77	0.55	1.04	0.48
04620A1-1-7-4	42.6	30.3	53.0	27.9	50.5	59.5	41.7	53.7	0.84	0.51	1.32	0.52
04719A1-16-1-1-7	40.4	27.8	49.5	25.7	49.4	56.2	47.4	49.4	0.82	0.50	1.04	0.52
05219A1-8-21-2-4	40.7	28.6	47.9	30.7	52.1	54.1	43.0	45.5	0.78	0.53	1.16	0.67
05222A1-1-2-1	38.9	29.4	52.9	27.3	50.1	54.5	43.7	47.6	0.78	0.54	1.23	0.57
0537A1-3-12	41.5	33.4	64.9	31.8	51.4	57.4	49.0	53.0	0.81	0.58	1.32	0.62
07290A1-12	36.7	30.0	60.9	26.7	53.1	58.4	49.6	48.9	0.69	0.51	1.23	0.55
ALLEGIANCE	39.3	27.0	62.5	32.2	56.2	55.2	46.7	56.6	0.70	0.49	1.34	0.57
FOSTER	40.6	29.4	65.3	31.4	53.2	57.4	51.1	50.0	0.76	0.51	1.28	0.63
IL01-11934	48.8	30.9	72.0	36.5	57.9	52.7	52.4	56.7	0.84	0.59	1.36	0.64
IL06-13072	45.7	32.4	58.0	32.5	55.3	61.2	49.1	58.3	0.83	0.53	1.19	0.56
IL06-7550	42.5	32.9	62.0	33.6	53.6	64.0	50.7	55.6	0.79	0.51	1.22	0.61
IL07-19334	42.4	32.9	60.9	25.6	55.7	61.4	47.9	56.2	0.76	0.54	1.27	0.45
IL07-20728	39.3	32.0	60.3	40.8	53.7	59.1	50.1	58.0	0.73	0.54	1.21	0.71
IL07-20743	51.3	31.3	69.0	30.8	52.2	59.0	51.9	56.0	0.98	0.53	1.33	0.50
IL07-21847	43.9	27.7	67.8	31.7	53.3	55.5	50.3	57.2	0.82	0.50	1.35	0.56
IL07-23420	38.3	30.3	73.9	33.7	53.2	58.3	50.0	53.4	0.72	0.52	1.48	0.64
IL07-6861	50.6	30.0	62.5	30.3	58.7	57.5	52.7	46.6	0.86	0.52	1.19	0.65
IL08-34020	46.7	30.5	50.2	22.6	58.1	57.9	47.9	51.7	0.80	0.53	1.05	0.44
IL99-26442	41.8	27.1	74.4	30.0	50.2	59.7	52.9	54.2	0.83	0.45	1.41	0.55
KY02C-1058-03	46.6	27.6	55.9	28.8	55.4	57.8	42.9	50.9	0.84	0.48	1.31	0.58
KY02C-1076-07	41.1	29.7	66.5	30.3	52.1	59.6	52.4	54.6	0.79	0.50	1.27	0.55
KY02C-1121-11	32.0	27.2	72.7	37.3	46.6	55.4	54.7	50.6	0.69	0.49	1.33	0.73
KY02C-1121-75	47.3	28.9	78.9	37.1	53.5	61.5	52.4	60.0	0.88	0.47	1.51	0.62
KY02C-1122-06	39.0	31.4	56.9	29.6	51.9	64.7	55.5	54.2	0.75	0.49	1.02	0.55
KY02C-2215-02	43.3	32.0	65.2	33.3	54.6	59.2	48.2	56.7	0.79	0.54	1.36	0.59
KY02C-3004-07	39.5	27.1	64.6	35.6	53.3	57.5	44.6	50.5	0.74	0.47	1.45	0.71
KY02C-3005-25	48.9	30.4	65.4	28.8	55.8	59.0	55.1	53.9	0.88	0.52	1.18	0.54
KY03C-1002-02	48.4	33.7	61.9	33.7	52.3	59.9	48.9	50.3	0.93	0.56	1.26	0.67
KY03C-1192-37	47.6	30.7	65.8	29.2	55.0	56.9	51.8	53.5	0.87	0.54	1.27	0.54
KY03C-1195-10-1-5	40.5	28.4	68.5	29.8	52.7	59.8	49.0	57.2	0.77	0.47	1.40	0.52
KY03C-1221-01	40.2	28.2	49.9	24.9	51.5	53.7	51.3	46.4	0.78	0.52	1.05	0.54
KY03C-1221-06	39.4	27.4	63.7	25.0	50.5	53.3	45.7	49.4	0.78	0.51	1.39	0.51
KY03C-1221-22	40.9	29.1	51.8	26.6	53.7	57.7	44.9	52.0	0.76	0.50	1.15	0.51
KY03C-1237-01	43.3	29.1	69.9	38.0	56.8	57.6	46.9	53.6	0.76	0.50	1.49	0.72
KY03C-1237-15	46.9	27.4	58.8	30.4	51.5	59.8	42.5	54.5	0.91	0.46	1.38	0.56
KY03C-1237-32	45.7	28.4	63.4	29.0	51.2	62.1	45.1	54.5	0.89	0.46	1.41	0.53
KY03C-2047-02	43.8	30.3	62.0	31.1	51.5	54.3	52.5	55.7	0.85	0.56	1.18	0.57
KY03C-2047-06	42.2	31.3	65.4	29.8	53.4	56.3	49.6	58.9	0.79	0.55	1.32	0.51
KY03C-2049-02	35.3	26.0	75.0	26.9	46.9	59.0	53.0	52.6	0.75	0.44	1.41	0.51
KY03C-2314-08	48.8	30.4	72.9	40.4	56.0	61.2	56.8	57.3	0.87	0.50	1.28	0.71
KY03C-2399-02	32.8	31.6	60.8	28.0	50.7	62.8	43.7	58.9	0.65	0.50	1.41	0.48
KY04C-1128-38-1-5	43.8	29.9	66.5	26.9	55.4	55.9	44.4	58.1	0.79	0.53	1.43	0.46
KY04C-2006-41-1-1	39.4	32.2	77.3	33.1	52.2	56.7	52.8	59.1	0.75	0.57	1.47	0.56
KY04C-2151-40	42.1	29.5	59.7	31.5	50.1	55.9	44.6	50.9	0.84	0.53	1.34	0.62
KY04C-2151-41	41.4	27.3	57.6	31.1	46.6	55.4	46.6	50.9	0.89	0.49	1.24	0.61
KY04C-3006-33-14-3	38.1	24.5	67.8	33.6	52.9	56.8	44.4	56.1	0.72	0.43	1.53	0.60
KY05C-1007-2-12-5	43.1	31.1	68.2	36.3	51.6	61.5	51.0	58.2	0.83	0.51	1.34	0.62
KY05C-1105-42-20-1	38.3	27.3	59.2	36.1	48.0	56.4	52.1	57.9	0.80	0.48	1.14	0.62
KY05C-1381-77-7-5	43.0	27.8	65.6	33.4	54.1	56.5	50.2	55.6	0.79	0.49	1.31	0.60
KY05C-1617-17-17-3	43.2	30.1	71.7	29.6	52.1	58.0	50.2	58.1	0.83	0.52	1.43	0.51
KY06C-1003-139-8-3	47.2	31.2	70.1	34.3	52.8	57.3	49.3	56.7	0.89	0.55	1.42	0.60
KY93C-1238-17-1	44.0	33.2	80.0	35.0	56.7	57.5	52.7	57.8	0.78	0.58	1.52	0.61
PEMBROKE	35.4	30.4	72.4	33.7	47.6	61.0	50.8	57.4	0.74	0.50	1.42	0.59
$\bar{x}$	42.1	29.6	63.4	31.1	52.7	57.7	48.9	53.9	0.80	0.51	1.30	0.58
SE	0.57	0.28	1.09	0.54	0.37	0.40	0.50	0.52	0.01	0.005	0.02	0.01

Chapter 6

Breeding for Nitrogen Use Efficiency to Combat Heat Stress Caused by Climate Change

Introduction

Winter wheat (*Triticuum aestivum* L.) is an important component of the national and global food supply. As human population growth continues, worldwide demand for wheat will continue to increase. However, the productivity of wheat and other crops, worldwide, is being threatened by global climate change. Climate models have predicted that temperatures will rise 1.4-5.8^{0}C over the next 30-50 [Intergovernmental Panel on Climate Change (IPCC), 2001 and 2007](Keating *et al.*, 2010). In wheat, temperatures greater than 14^{0}C stress the crop causing plant photosynthesis rate to decrease (Prasad *et al.*, 2008). Future environments will also be subjected to increased temperature variability and a greater number of hot days (Farooq *et al.*, 2011). Studies using a crop model linked to field data showed that for every 1^{0}C increase in global mean temperature wheat production would decrease 6%, resulting in a 42 Mt loss of wheat with each degree temperature increase (Nelson *et al.*, 2009; Nelson *et al.*, 2014; Van Ittersum *et al.*, 2003; Asseng *et al.*, 2014). Overall, grain yields were predicted to decline in most regions, worldwide, and temperature impacts may be greater and begin sooner than previously thought (Asseng *et al.*, 2014; Challinor *et al.*, 2014). In order to create new genotypes adapted to future climates, greater understanding of how crops act in response to elevated temperatures and how heat stress tolerance can be enhanced is a necessity (Farooq *et al.*, 2011).

Higher temperatures between anthesis and grain maturity cause grain yield to decrease because there is less time to move assimilates to the grain. Both components of yield, grain number and grain weight, are susceptible to increased temperature (Ferris *et al.*, 1998). The developmental stage at which elevated temperatures occur will determine which component of grain yield will be affected. For example, during anthesis, temperatures above 20^{0}C may considerably decrease grain number per spike (Saini and

Aspinall, 1982). Heat stress accelerates development of the spike, reducing spikelet number, resulting in fewer grains per spike (Saini and Aspinall, 1982; Porter and Gawith, 1999; Farooq *et al.*, 2011). The most sensitive stage during reproductive growth is between the double ridge (appearance of double ridges on apex of shoot) and flag leaf stage. During this time, florets are produced in the spikelets that form in the spike. The elevated temperatures hasten development during this period of time, causing spikelet number per spike and grain number per spikelet to decline (McMaster, 1997). Grain number can also be reduced during floral initiation. For instance, Fischer (1985) found that for every one increment temperature increase (°C), grain number per spike decreased by 4% in the 30 days preceding anthesis. Because insufficient assimilates can cause the floret number to decline, assimilate availability can influence floret development and thus, grain number (Abbate *et al.*, 1995; Demotes-Mainard and Jeuffroy, 2004). Heat stress tolerance in terms of the effect on wheat grain number and size varies among the genotypes (Farooq *et al.*, 2011). For example, one experiment studying the influence of grain characteristics in spring wheat genotypes found that 14 varieties exhibited smaller grain size when exposed to elevated temperatures, no matter how long or at what developmental stage the event occurred (Castro *et al.*, 2007). Overall, rising temperatures reduce spikelet fertility, grains per spike, grain size, and quality.

One of the main factors that influence grain yield and quality is nitrogen (N). Nitrogen is a critical nutrient for canopy growth, and canopy photosynthesis drives grain yield and quality. However, adding N fertilizer to the soil to enhance N uptake by wheat experiencing heat stress may not be a viable option for growers. Excess N has been shown to have adverse environmental impacts, such as eutrophication of freshwater and marine ecosystems that occurs when high quantities of N fertilizer are added to soil and then washed into the stream through runoff. Therefore, greater knowledge is pertinent to understand how mechanisms associated with nitrogen-use efficiency (NUE) work under climatic stress, specifically higher temperatures. Breeding for NUE is thought to be a possible strategy to develop wheat lines adapted to warmer environments because these genotypes are able to take up and store more N in stem reserves. When the photosynthetic capacity of the plant is compromised due to elevated temperatures, the plant can utilize N stored in stem reserves to continue grain filling and produce yield

(Farooq *et al.*, 2011). Adaptation to heat stress has been shown to be related to the plants ability to accumulate stem reserves prior to anthesis (Blum *et al.*, 1994; Farooq *et al.*, 2011). There is evidence of genotypic variation for assimilate contribution to grain filling under heat stress (Yang *et al.*, 2002; Farooq *et al*, 2011). Variation in NUE in wheat has been documented and is likely to vary under elevated temperatures as well.

Nitrogen use efficiency is the yield of grain per unit of available N in the soil (including residual soil N and fertilizer added). There are two components of NUE: uptake efficiency (NUpE; capability of plant to remove N from the soil as nitrate and ammonium ions) and utilization efficiency (NUtE; capability to use N to generate grain yield). Several studies have examined the effects of increased temperature on N uptake and allocation in some crop species (Jonassona *et al.* 2004; An *et al.* 2005; Yang *et al.* 2011). For example, a night-time warming field experiment using reflective curtains showed that N accumulation in winter wheat during anthesis was 17-43% higher in the warmed than the unwarmed treatment. However, N utilization efficiency was decreased in the warmed treatment causing reduced N allocation towards yield during grain filling, resulting in a 6-25% yield decrease (Zhang *et al.* 2013). Total plant N at anthesis and maturity, along with grain N has been shown to decrease under dryer conditions due to a lower NUpE and subsequent NUtE, especially at higher N levels (Giuliani *et al.*, 2011). Researchers and breeders must continue to develop a better understanding of the fundamental mechanisms and traits associated with N uptake and utilization efficiency under warmed environments. Therefore, 40 soft red winter wheat genotypes were actively warmed at Lexington, KY to identify genotypes that have high NUE under both warmed and control environments and to identify traits that may be associated with NUE and tolerance to warmer climates.

Materials and Methods

Site Description and Experimental Design

The study was conducted at University of Kentucky Spindletop Research Farm in Lexington, KY (38^{0}7’37.81’’N, 84^{0}29 44.85’’ W) (37^{0}6’7.37’’ N, 87^{0}52’13.62’’W). The soil at the site was characterized by a Maury silt loam [fine, mixed, semiactive,

mesic Typic Paleudalfs] soil. The experimental material consisted of 40 winter wheat genotypes which were a subset of the 56 TCAP (http://www.triticeaecap.org/) nested association mapping panel (NAM) entries grown in the N study. The genotypes differed in characteristics such as heading date, height, and the environment in which the lines were adapted. Therefore, these lines represented the diversity within the winter wheat population and were likely to vary in other traits such as grain protein and nitrogen-use efficiency (NUE).

Forty soft red winter wheat lines were planted on 29 October 2013 in a randomized complete block design under warmed and unwarmed environments at Spindletop farm in Lexington, KY. The experimental unit was a headrow 1.5 m in length at a row spacing of 17.8 cm. Two reps per genotype per treatment were planted. 112 kg N ha^{-1} was applied in a 34 kg N ha^{-1} and 78 kg N ha^{-1} split on 13 March and 19 April 2014, respectively. Soil heating cables were used to simulate climate change effects in the warmed environment. Cables were inserted and buried at a depth of 2.54 cm on 15 November 2013 after planting. A Campbell weather station was placed at the site to measure soil temperature and air temperature within each treatment. To measure soil temperature, 16 probes were placed in the warmed treatment and 4 in the control treatment that measured. Soil temperature probes were placed at a depth of 10 cm. To measure air temperature, 4 probes were placed in the warmed treatment and 2 in the control. Each air temperature probe was positioned on the center of a C-shaped metal bar placed into the ground, head of the probe facing upward, 10 cm above the ground. Each probe within each treatment measured soil/air temperature every 15 minutes throughout the duration of the study. Two soil moisture probes were also placed in each treatment, at a 10 cm depth, to measure percent soil moisture content for the duration of the study.

Field sampling and Data Collection

Soil Sampling

Soil samples were collected prior to N application in each treatment. Within each treatment, six soil cores were taken to a 30.48 cm depth with a 1.6 cm diameter soil probe. The cores collected from each treatment were mixed in a plastic bucket by hand,

air dried, and placed in paper soil bags. Soil samples were ground to a fine powder using a soil grinder. The grinder was cleaned with an air compressor in between samples. Soil samples were also collected when plants had reached anthesis for each treatment following the same protocol for the pre-N application soil sampling.

Agronomic Traits and N sampling

For each headrow, heading and anthesis dates were recorded. Heading date was determined when 50% of the spikes in a row had emerged from the flag leaf sheath, or boot. Anthesis date was recorded when 50% of the plants in a row were showing visible anthers. Row length and row height were measured in each plot at the soft dough stage. The soft dough stage is equivalent to physiological maturity, which is when maximum dry matter accumulation has occurred and kernels have turned a buff color. A SPAD meter was used to measure N status differences at anthesis and physiological maturity for each genotype within each treatment by calculating the chlorophyll content index (CCI). The SPAD meter was placed on the center of five flag leaves and an average CCI was taken for each genotype within each treatment.

At anthesis, a 20 cm segment of row was hand cut at the soil surface from 12 genotypes within each treatment to provide a whole plant sample for subsequent analysis. These 12 genotypes were chosen because theses lines varied in heading date, height, and other agronomic traits. Thus, these lines were expected to differ in root biomass and vegetative N at anthesis. The sample was air dried in the greenhouse. The roots from the cut 20 cm section of row were excavated with a trowel at a depth of 10 cm (where main root mass is located in wheat) and frozen. Root samples from the 12 selected genotypes were thawed overnight, washed over a 53 μm sieve, dried at 55^0C in an oven overnight, and weighed. At harvest maturity, the entire length of the headrow within each treatment was hand harvested using a sickle. Harvest maturity of a row was determined when grain was hard and could not be split by thumbnail. Plants were cut as close to the soil surface as possible to collect all above-ground plant vegetative tissue. Maturity samples were air dried in greenhouse. Head number and head length were recorded from vegetative material collected at anthesis. Plant number and vegetative biomass was

recorded from both anthesis and maturity biomass from each replication in each environment. After harvest, grain was mechanically threshed and yield was measured.

Data Processing and Analysis

Soil N analysis

Soil samples collected within each treatment were extracted for ammonium and nitrate using the KCl method. Ten grams of soil from each sample was shaken for 30 minutes in 25 mL of 2 mol KCl (150 g KCl per 1000 mL of deionized (DI) water) at 200 rpm. Then, 1 mL liquid extract from each sample was pipetted (a new pipette head was used per sample) into cluster tubes, recording the order samples were arranged in the cluster tube box so that the data could be associated with the correct sample once analysis was complete. The cluster tube box with samples was centrifuged for 27 minutes and 15 µL of calibration standards and samples was pippetted into the wells of two microplates, one for nitrate analysis and one for ammonium analysis.

To prepare the nitrate microplate for analysis, 200 µL of 8.5 pH ammonium buffer was added to each nitrate microplate well. A copperized cadmium reductor was placed into each well of the microplate and shaken for 60 minutes on a titer plate shaker to convert nitrate to nitrite (Nydhal, 1976; Crutchfield and Grove, 2011). The Griess reaction was used to colormetrically measure the nitrite concentration within each sample. To induce the Griess reaction, 60 µL of Griess reagent (4 mL of 1.0 % sulfanilamide in 3 N hydrochloric acid and 4 mL of 0.1 % N-(1-Naphthyl)) was added to each well of the microplate (Griess, 1858; Crutchfield and Grove, 2011). The microplate was inserted into the Microplate Versa Max Analyzer and nitrite levels within each well were read at 542 nm (Henriksen and Selmer-Olsen, 1970; Crutchfield and Grove, 2011).

The ammonium microplate was colormetrically measured using a modified Berthelot reaction. The Berthelot reaction was induced by inserting 100 µL of sodium hydroxide-hypochlorite and 100 µL of phenol-nitroprusside into each microplate well (Berthelot, 1859; Chaney and Marbach, 1962; Weatherburn, 1967; Ngo *et al.*, 1982). Then the microplate was shaken on a titer plate shaker for 45 minutes. Afterwards, the

microplate was inserted into the Microplate Versa Max Analyzer and ammonium levels were measured at 630 nm.

Measuring N Traits

Whole grain samples were placed under the Near Infrared reflectance (NIR) (DA7200; 950-1650 nm wavelength range; manufactured by Perten instruments) instrument to measure percent grain protein. Grain protein content was converted to percent N by using the conversion factor for grain, 6.25. Anthesis and maturity sampling of vegetative material were ground to a fine powder using a UDY cyclone mill, and then run under the NIR instrument to measure % N content. Total plant N uptake (kg ha^{-1}) was determined by summing grain N (kg ha^{-1}) (yield*%N) and N in vegetative biomass at maturity (kg ha^{-1}) (biomass *% vegetative N). Nitrogen use efficiency and NUE components were calculated as follows: nitrogen-use efficiency (NUE)=yield/soil N supply (Pre-N soil N and fertilizer N), nitrogen uptake efficiency (NUpE) = Total plant N/ soil N supply (Pre-N application soil and fertilizer N), nitrogen utilization efficiency (kg yield kg^{-1} plant N) (NUtE) =yield/Total plant N.

Statistical Analysis

Analysis of variance (ANOVA) was performed using the General Linear Models procedure (Proc GLM; SAS 2002) to determine genotype and environment effects. The model used was:

$$Y_{ijk} = \mu + ENV_i + R(ENV)_{ij} + G_k + ENV_i * G_k + E_{ijk};$$

where Y_{ijk} = the observation in the *k*th genotype in the *j*th rep in the *i*th environment, μ = the overall mean, $R(ENV)_{ij}$ = the effect of *j*th rep within *i*th environment, $ENV_i * G_k$ = the effect of the interaction of the *i*th environment and the *k*th genotype, and E_{ijk} = the residual error. LSMEANS were computed to measure treatment differences among genotypes.

Broad sense heritability of N traits was estimated on an entry mean basis using the following model:

$Y_{ijk} = \mu + ENV_i + R(ENV)_{ij} + G_k + ENV_i * G_k + E_{ijk}$;

where Y_{ijk} = the observation in the *k*th genotype in the *j*th rep in the *i*th environment, μ = the overall mean, G_k = the effect of the *k*th genotype, $R(ENV)_{ij}$ = the effect of *j*th rep within *i*th environment, ENVi * G_k = the effect of the interaction of the *i*th environment with the *k*th genotype, and E_{ijk} = the residual error.

Agronomic and N trait data was analyzed using the General Linear Models procedure (Proc GLM; SAS 2002). Genotypic and phenotypic variances were estimated from the expected mean squares (EMS) and heritability estimates were computed as:

$h^2 = Vg/Vp$

where h^2 = heritability, Vg = genotypic variance, Vp = phenotypic variance.

Confidence intervals (90 %) were calculated after Knapp *et al.* (1985) as:

UL= 1- [MS3/MS2 * FUL (.10, v1 and v2 df)]$^{-1}$ and

LL= 1- [MS3/MS2 * FLL (.90, v1 and v2 df)]$^{-1}$;

where UL = upper limit of the confidence interval, MS3 = entry mean square, MS2 = residual mean square, FUL and FLL = F value for the upper and lower limits calculated using the FINV function of Microsoft Excel (2007), respectively.

Proc CORR (SAS 2002) was used to analyze the relationship among traits on an entry mean basis.

Association Mapping in TASSEL

All entries in the mapping panel were genotyped with the 9K Illumina SNP chip to identify single nucleotide polymorphisms (SNP) associated with the traits measured during the course of the study. TASSEL (http://www.maizegenetics.net) software was used to carry out association mapping. The Q+K method was implemented as a mixed linear model to determine association of the N traits and agronomic traits measured with QTL markers. The statistical model used was described as:

$$Y = Xb + Zu + e;$$

where y is the vector of observations; b is an unknown vector containing fixed effects including genetic marker and population structure (Q); u is an unknown vector of random additive genetic effects from multiple background QTL for individuals or lines; X and Z are the known design matrices; and e is the unobserved vector of random residuals (Bradbury *et al.* 2007).

Principal Component Analysis

The Proc Princomp procedure was performed in SAS to generate a principal component analysis (PCA) for both control and warmed treatments. The PCA was used to reduce the observed variables into a smaller number of principal components (artificial variables) that would account for most of the variance detected in the data (Yeater *et al.*, 2014). Within the procedure, the principal axis method was used to extract the components, and this was followed by a varimax (orthogonal) rotation. The PC's with the largest eigenvalues were selected and plotted for each treatment (Yeater *et al.*, 2014).

Results and Discussion

Effect of Warming on Agronomic and N Traits

There was no significant difference in genotypic variation between environments for the agronomic traits or N traits, except NHI ($p<0.05$), though HI was significant at $P<0.10$ (Table 6.1). This may have been due to the choice of headrows as experimental units. Previous research in our lab has shown that experimental error associated with headrows is very large (D. Van Sanford, personal communication 2015). There was no G*E interaction for any trait measured. However, many traits were significantly different between environments (Tables 6.1 and 6.2). Temperature in the warmed environment were consistently higher than in the control. Under warming, soil temperatures were 3-5°C, and air temperatures were 1-2°C, higher than in the control (Figures 6.1 and 6.2). In the warmed environment, soil moisture was 1-2% lower than in the control (Table 6.3).

Elevated temperatures caused development in the warmed treatment to accelerate. Heading date and anthesis date occurred almost 5 days earlier in the warmed treatment

than in the control treatment (Table 6.1). Maturity date occurred about a week earlier in the warmed than in the control treatment, resulting in a shortened grain filling period in the warmed treatment (Table 6.1). Grain filling period (estimated as number of days between anthesis and physiological maturity) was 33 days in the control treatment and 29 days in the warmed treatment. Due to accelerated development, the plants in the warmed treatment were shorter, had lower yields, and had lower grain N (Table 6.1 and 6.2). This result is similar to other studies where warming was found to hasten phenology, reducing plant growth and yield. Several studies have indicated that elevated temperatures shorten key developmental stages, accelerating senescence and causing crop growth to decrease (Lavalle *et al.*, 2009; Hatfield *et al.*, 2011; Grant *et al.* 2011). Warming has also been shown to reduce seed set number and yield by causing floret fertility to decrease, along with grain filling period (Gibson and Paulsen, 1999; Baker, 2004; Wheeler *et al.*, 2009). Nitrogen uptake efficiency, N concentration at anthesis, and total plant N uptake were not significantly different between treatments (Table 6.2). This is likely because both treatments had the same N supply (143.2 kg ha^{-1}, estimated as fertilizer added plus residual soil N) and were not N limited (Table 6.2). Nitrogen utilization efficiency and NUE were lower in the warmed than control treatment. This may be related to developmental differences: increased development may have reduced the length of time allowed for N remobilization to the grain, causing more N to be left in the biomass, thus causing a reduction in yield and grain N removal (Table 6.1 and 6.2).

Traits Associated with NUE

Association mapping was conducted to identify QTL associated with NUE under warmer environments. In the warming study, there were no traits that were significant at 0.05 or 0.01 after the Bonferroni multiple test correction (0.05/21067; 0.01/21067), $2.4*10^{-6}$; $4.7*10^{-7}$. The numerator is the *p*-value being tested and the denominator in the Bonferroni correction is the number of SNPs being tested. The Bonferroni correction adjusts *p* values when multiple dependent or independent statistical tests are being performed concurrently on a single data set. The Bonferroni correction is performed by dividing the critical *p* value (0.05, 0.01) by the number of comparisons being made, in this case the number of SNP's (21067). The modified *p* value ($2.4*10^{-6}$; $4.7*10^{-7}$) is then

used to test the statistical power of the data (Bradbury *et al.*, 2007). The markers with the strongest p-values in the warmed were BS00000244_51, BS00009777_51, and BS00004221_51 for CCI at harvest maturity. The markers with strongest p-values in the control were BS00009295_51 for heading date; BS00009777_51, BS00011516_51, BS00011630_51 for height; BS00005034_51 for grain protein; and BS00005034_51 for NUtE and N in straw at maturity. The lack of significant markers is probably due to few number of lines used to perform the analysis. Most association analyses require a much larger set of lines for mapping, 150 lines at minimum (Crossa *et al.*, 2007; von Zitzewitz *et al.*, 2011) (Table 6.4).

Association mapping in TASSEL did not shed light on SNP's associated with NUE or NUE components. From the study, height was significantly associated with NUtE and NUE ($p<0.01$) in each environment (Tables 6.5). However, these correlations were not particularly strong. Also, heritability estimates were low for all N traits measured (Table 6.6). Identifying easily quantifiable traits related to NUE would be very beneficial to breeding programs. There are many traits that influence nutrient efficiency, but these traits are not easy to identify as these traits are very much influenced by environmental interaction. To gain further insight into the mechanisms controlling NUE under warmed environments and to elucidate plant response to warming, other tools such as ecophysiological models and crop models could be beneficial (Asseng *et al.*, 2014; Dresbøll and Thorup-Kristensen, 2014.). Also, identifying QTL's for NUE and NUE components using molecular methods and association mapping could improve development of N use efficiency, as was done in oilseed rape (Bouchet *et al.*, 2014) or barley (Kindu *et al*, 2014.). Further research is incorporating genetics and physiology could help discover the location of QTL linked to NUtE and NUpE to better understand the way in which alleles associated with QTL may influence differences in NUE in winter wheat (Bueren *et al.* 2014).

Data Structure Observed from Principal Components

The principal component analysis (PCA) is a variable reduction procedure used to determine the underlying structure in the data. The PCA explains the direction where the most variance is observed. When the PCA is performed the eigenvector and eigenvalues

of the data set are determined. The eigenvector is the direction in which the variation in the data is occurring and the eigenvalue explains how much variation there is in the data set in that particular direction. The largest eigenvectors with the greatest eigenvalues are the principal components. The PCA showed that only the first two components displayed eigenvalues greater than 1, suggesting that only the first two components were significant. Principal component 1 (PC 1) explains 73.3% of the variation. The traits contributing to the variation of PC1 are yield, N grain, total plant N, NUpE, and NUE. Principal component 2 (PC 2) explains 22.5% of the variation. The traits contributing to the variation of PC2 are N in straw at maturity and NUtE (Table 6.7 and 6.8). In both cases, PC 1 seems to be related to uptake efficiency and total plant N and PC 2 seems to be related to utilization efficiency and N remobilization. For PC 2, N in the straw at maturity had a positive correlation, 0.67, while the correlation to NUtE was negative, -0.67, in the control environment (Table 6.7). The opposite correlation was observed in the warmed environment, N in straw at maturity was -0.54 and NUtE was 0.74 (Table 6.8). This may be because in the warmed environment N content in the straw at maturity was higher than in the control and NUtE was lower in the warmed than control. Overall, less N was remobilized to the grain under warming.

Genotypic Performance under Warming and Implications for Breeding

Among the genotypes, IL-23420 had the highest NUE and yield in the control and 05219A1-8-21-2-4 in the warmed. The most stable genotypes across treatments in terms of yield and NUE were 05219A1-8-21-2-4 and KY03C-1195-10-1-5 (Tables 6.9 and 6.10). Both 05219A1-8-21-2-4 and KY03C-1195-10-1-5 actually increased yield and NUE under warming, along with grain N. This was due to an increase in uptake efficiency under warming (Table 6.9 and 6.10). In contrast, Foster, KY05C-1105-42-20-1, 04719A1-16-1-1-7, and IL07-19334 had the lowest yield, NUE, and grain N in the warmed environment due to a decrease in uptake efficiency under elevated temperatures (Tables 6.11 and 6.12). These lines exhibited some of the lowest uptake efficiencies and total plant N in the warmed treatment.

On average, utilization efficiency decreased under warming, but there were also some instances where NUtE did not change or increased (Tables 6.10 and 6.11). For

instance, NUtE in 04719A1-16-1-1-7 did not change under warming and was high in comparison to the other lines (43.2 kg ha^{-1}), but this line experienced the lowest NUpE and total plant N (0.68 kg kg^{-1}, 96 kg ha^{-1} respectively) under warming, thus resulting in low NUE and yield in higher temperatures (Tables 6.11 and 6.12). Allegiance, Pembroke and KY03C-1237-01 decreased in yield, NUE, and grain N in the warmed treatment. This seems to be due to a large decrease in utilization efficiency rather than uptake efficiency, which did not change significantly between treatments for these genotypes (Tables 6.9, 6.10, 6.11, and 6.12). There were 15 genotypes in which yield, NUE, and grain N content was increased in the warmed treatment. Of those there were 9 in which increased yield could be associated with increases in uptake efficiency under warming, genotypes such as 05219A1-8-21-2-4, KY03C-1237-32, IL06-7550, KY04C-2151-40, and KY02C-3005-25 (Table 6.9, 6.10, 6.11, and 6.12). Even though there was no difference in root biomass between N treatments (Table 6.1) there is a possibility that these lines may have more extensive root morphology and are able to take up more N under warming. Studies have indicated that root architecture, rather than root biomass, may be of greater importance to plant N uptake. These traits could include root length, rooting depth, root growth rate, root hair density, and root hair length (Lynch *et al.*, 2014).

There were 4 genotypes (KY03C-2047-06, KY03C-1221-01, KY04C-3006-33-14-3, 0537A1-3-12) that increased genotypic performance under warming due to increased NUtE when exposed to elevated temperatures, while NUpE remained the same or changed little. The other 2 lines (04620A1-1-7-4 and KY04C-1128-38-1-5) increased yield, NUE, and grain N due to an increase in both NUpE and NUtE in warmed treatment (Tables 6.9, 6.10, 6.11, and 6.12). Even though NUpE was not significantly different between treatments, NUpE had a stronger correlation to yield and grain N in both treatments compared to NUtE (Table 6.5). This suggests that NUpE may be more important in producing yield and grain N in future warmer conditions in Kentucky. Selecting for NUpE and traits related to NUpE, such as total plant N may be a good strategy to increase overall NUE, yield, and grain N. Lines that have a higher NUpE may be able maintain yield and quality under warmer environments. These cultivars can be incorporated into breeding programs developing genotypes adapted to future climates.

Table 6.1. LSMEANS of controlled and warmed environments (E) for agronomic traits in a warming study of 40 soft red winter wheat genotypes from the warming study 2014 Lexington, KY calculated from the ANOVA. Environment (E), rep (R), genotype (G). Heading date (HD) (May 1=1, May 2=2, etc.), anthesis date (AD) (May 1=1, May 2=2, etc.), maturity date (MD) (June 1=1, June 2=2, etc.), height (H) (cm), chlorophyll content index anthesis (CCIa), chlorophyll content maturity (CCIm), plant number (PN), vegetative biomass at maturity (Vbm) (kg ha^{-1}), yield (Y) (kg ha^{-1}), and harvest index (HI), RB (root biomass).

E	HD	AD	MD	H	CCIa	CCIm	PN	Vbm	Y	HI	RB
Control	9.3	12.5	14.5	81.0	44.8	27.7	149.9	6512.2	6995.1	0.52	5.1
Warmed	5.9	8.5	6.5	75.2	45.3	19.1	136.1	6785.3	6103.2	0.48	5.3
E	<0.0001	<0.0001	<0.0001	<0.0001	0.50	<0.0001	0.04	0.32	0.003	<0.0001	0.74
R(E)	0.98	0.07	0.07	0.18	0.10	<0.0001	0.06	0.01	0.01	0.96	0.18
G	0.23	0.77	0.82	0.81	0.83	0.35	0.23	0.27	0.62	0.08	0.16
G*E	0.92	0.54	0.54	0.17	0.13	0.78	0.44	0.58	0.34	0.38	0.12

Table 6.2. Averages calculated from LSMEANS for controlled and warmed environments (E) differences for nitrogen traits for the 40 soft red winter wheat genotypes calculated from the ANOVA for the warming study 2014 Lexington, KY. Environment (E), rep (R), genotype (G). % grain protein (Gp), % N at anthesis (Na), % N at maturity (Nm), nitrogen harvest index (NHI), N utilization efficiency (NUtE) (kg yield kg^{-1} plant N), N uptake efficiency (NUpE), N use efficiency (NUE).

E	Gp	Na	Nm	Vnm	Ngc	TN	NHI	NUtE	NUpE	NUE
Control	11.6	1.79	0.49	31.9	129.8	161.6	0.81	43.7	1.13	48.9
Warmed	11.5	1.74	0.66	45.7	111.6	157.3	0.71	39.4	1.1	42.6
E	0.15	0.20	<0.0001	<0.0001	0.001	0.55	<0.0001	<0.0001	0.55	0.003
R(E)	<0.0001	0.49	<0.0001	<0.0001	<0.0001	<0.0001	0.001	<0.0001	<0.0001	0.006
G	0.45	0.21	0.81	0.05	0.80	0.65	0.03	0.43	0.65	0.62
G*E	0.25	0.22	0.84	0.58	0.61	0.82	0.17	0.07	0.82	0.34

Table 6.3. Average monthly percent soil moisture measured from soil moisture probes placed at a depth of 10 cm, two reps per treatment. Soil moisture was measured daily every hour from March to June from each probe. ANOVA was performed to determine significant differences between monthly averages. Significance level for the following effects in the model shown in lower half of table: N environment (E), rep (R), and month (M).

Month	Control	Warmed
Mar	9.5	8.7
April	17.6	16.3
May	16.1	14.8
Jun	6.4	6.3
	E	<0.0001
	R(E)	<0.0001
	M	<0.0001
	M*E	<0.0001

Table 6.4. Association mapping of agronomic traits and markers using the Mixed Linear Model in TASSEL of each N environment (E) from the warming study 2014 Lexington, KY.

E	Trait	Marker	Locus	Site	F	p	Marker R^2
Warmed	CCI at maturity	BS00000244_51	--	--	6.7	0.01	0.19
	CCI at maturity	BS00009777_51	--	--	4.9	0.03	0.14
	CCI at maturity	BS00004221_51	--	--	4.3	0.05	0.12
Control	Heading date	BS00009295_51	--	--	5.7	0.02	0.17
	Height	BS00009777_51	--	--	6.1	0.02	0.17
	Height	BS00011516_51	--	--	3.9	0.03	0.22
	Height	BS00011630_51	--	--	3.9	0.03	0.22
	Protein	BS00005034_51	--	--	5.5	0.01	0.31
	NUtE	BS00005034_51	--	--	4.3	0.02	0.23
	N content in straw at maturity	BS00005034_51	--	--	3.5	0.04	0.17

Table 6.5. Pearson correlations between N traits and agronomic traits for warming study 2014 Lexington, KY. Control treatment above diagonal, warmed treatment below. Heading date (HD), anthesis date (AD), Chlorophyll content index at anthesis (CCIa), height (H), plant number (PN), Vegetative biomass at maturity (Bvm), chlorophyll content index at maturity (CCIm), yield (Y), harvest index (HI), % grain protein (Gp), % N at maturity (Nm), Vegetative N content maturity (Vnm), N grain content (Ngc), nitrogen harvest index (NHI), total plant N (TN), nitrogen utilization efficiency (NUtE), nitrogen uptake efficiency (NUpE), nitrogen use efficiency (NUE).

	HD	AD	CCIa	H	PN	Vbm	CCIm	Y	HI	Gp	Nm	Nvm	Ngc	NHI	TN	NUtE	NUpE	NUE
HD	.	0.78**	-0.09	-0.03	0.03	-0.02	0.08	0.08	0.07	0.03	0.04	0.06	0.09	0.02	0.09	0.002	0.09	0.08
AD	0.52**	.	0.01	-0.04	0.001	-0.03	0.10	0.02	0.05	0.02	0.04	0.06	0.02	0.005	0.04	-0.0004	0.04	0.02
CCIa	0.10	0.07	.	0.03	-0.26	-0.21	-0.13	-0.22	0.07	0.19	-0.02	-0.20	-0.16	0.09	-0.20	-0.07	-0.20	-0.22
H	0.26	0.20	-0.12	.	0.39*	0.33*	-0.30	0.40*	-0.02	-0.46**	-0.54**	-0.10	0.29	0.31	0.21	0.53**	0.21	0.40**
PN	0.09	-0.04	0.46**	0.17	.	0.88**	-0.07	0.85**	-0.47**	-0.43**	-0.40**	0.51**	0.76**	-0.05	0.79**	0.26	0.79**	0.85**
Vbm	0.15	0.01	0.40*	0.10	0.75**	.	-0.11	0.84**	-0.67**	-0.29	-0.28	0.71**	0.79**	-0.25	0.87**	0.03	0.87**	0.84**
CCIm	-0.08	-0.03	0.33**	-0.14	0.07	0.09	.	-0.09	0.05	0.32**	0.31	0.13	-0.01	-0.15	0.03	-0.32**	0.03	-0.09
Y	0.24	0.21	0.37**	0.44**	0.73**	0.51**	0.04	.	-0.18	-0.32**	-0.48**	0.39*	0.96**	0.21	0.91**	0.36*	0.91**	1.00**
HI	-0.02	0.13	-0.19	0.26	-0.29	-0.70**	-0.11	0.22	.	0.08	-0.13	-0.74**	-0.15	0.73**	-0.35*	0.43**	-0.35*	-0.18
Gp	-0.20	-0.15	-0.17	-0.35*	-0.37*	-0.16	0.26	-0.50**	-0.21	.	0.44**	0.04	-0.03	-0.08	-0.02	-0.74**	-0.02	-0.32*
Nm	-0.22	-0.19	0.25	-0.44**	-0.19	-0.13	0.29	-0.41**	-0.20	0.43**	.	0.47**	-0.37*	-0.74**	-0.17	-0.81**	-0.17	-0.48**
Nvm	0.01	-0.13	0.48**	-0.08	0.61**	0.87**	0.23	0.28	-0.73**	0.04	0.36*	.	0.42**	-0.79**	0.66	-0.56**	0.66**	0.39*
Ngc	0.23	0.19	0.37*	0.40*	0.72**	0.53**	0.10	0.98**	0.20	-0.35*	-0.36*	0.32*	.	0.21	0.96**	0.16	0.96**	0.96**
NHI	0.09	0.22	-0.26	0.37*	-0.17	-0.54**	-0.18	0.34*	0.89**	-0.26	-0.59**	-0.78**	0.31*	.	-0.07	0.73**	-0.07	0.21
TN	0.17	0.09	0.50**	0.26	0.82**	0.80**	0.18	0.87**	-0.19	-0.24	-0.11	0.70**	0.90**	-0.13	.	-0.04	1.00**	0.91**
NUtE	0.15	0.23	-0.16	0.44**	-0.0005	-0.39*	-0.25	0.47**	0.82**	-0.59**	-0.66**	-0.67**	0.39*	0.93**	-0.01	.	-0.04	0.36**
NUpE	0.17	0.09	0.50**	0.26	0.82**	0.80**	0.18	0.87**	-0.19	-0.24	-0.11	0.70**	0.90**	-0.13	1.00**	-0.01	.	0.91**
NUE	0.24	0.21	0.37*	0.44**	0.73**	0.51**	0.04	1.00**	0.22	-0.50**	-0.41**	0.28	0.98**	0.34*	0.87**	0.47**	0.87**	.

*p<0.05; **p<0.01

Table 6.6. Broad sense heritability (h^2) and 90% confidence interval (upper limit (UL), and lower limit (LL)) for N traits across N environments and locations calculated from means squares from ANOVA. N grain content (Ngc), , % N maturity (Nm), vegetative N content maturity (Vnm), total plant N (TN), nitrogen harvest index (NHI), N utilization efficiency (NUtE), N uptake efficiency (NUpE), N use efficiency (NUE).

Trait	h2	LL	UL
Nm	0.04	0.36	-0.46
Vnm	0.39	0.09	0.60
Ngc	0.17	-0.78	0.25
TN	0.14	0.57	-0.31
NHI	0.23	-0.17	0.49
NUtE	0.42	0.32	0.55
NUpE	0.14	-0.31	0.43
NUE	0.22	-0.24	0.46

Table 6.7. Correlation matrix between eigenvectors and N traits calculated from principal component analysis in the control treatment of the warming study 2014 at Lexington, KY.

Trait	Principal Component 1	Principal Component 2
Yield	0.43	-.13
N grain content	0.43	-.06
N content in straw at maturity	0.18	0.67
Total Plant N	0.43	0.16
NUtE	0.19	-.67
NUpE	0.43	0.17
NUE	0.43	-.13
Eigen values	5.1	1.6
Total variance %	73.3	22.5

Table 6.8. Correlation matrix between eigenvectors and N traits calculated from principal component analysis in the warmed treatment of the warming study 2014 at Lexington, KY.

Trait	Principal Component 1	Principal Component 2
Yield	0.42	0.22
N grain content	0.43	0.16
N content in straw at maturity	0.30	-.54
Total Plant N	0.43	-.15
NUtE	0.05	0.74
NUpE	0.43	-.15
NUE	0.42	0.22
Eigen values	5.1	1.8
Total variance %	73.6	25.0

Table 6.9. LSMEANS of agronomic traits measured for each of the 40 soft red winter wheat genotypes for the control treatment from the warming study Lexington, KY 2014. Mean ($\bar{x}$), coefficient of variance (CV), standard error (SE) from ANOVA represented below. Genotypes are ranked based on yield.

Name	Heading date (May)	Anthesis date (May)	Maturity date (June)	Height (cm)	CCI at anthesis	CCI at maturity	Plant number	Biomass (kg ha^{-1})	Yield (kg ha^{-1})	HI (%)	Rank based on yield
IL07-23420	9.5	12.5	14.5	82.6	51.0	25.0	199	9595.6	9744.5	50.4	1
KY05C-1105-42-20-1	9	12	14	86.4	42.3	20.9	224.5	8570.0	9524.5	52.6	2
IL07-20728	9	12.5	14.5	78.7	40.8	28.7	201.5	8916.2	9359.7	51.2	3
IL07-19334	9	12.5	14.5	80.0	44.7	32.5	206	7510.3	9205.5	55.1	4
IL07-21847	9	12	14	82.6	43.7	22.9	148	6553.1	8803.5	57.3	5
07290A1-12	10	13	15	87.6	47.7	30.8	176.5	7258.4	8734.4	54.6	6
KY03C-1237-01	9	13	15	85.1	45.4	27.4	168.5	8380.6	8641.8	50.8	7
05219A1-8-21-2-4	10	13.5	15.5	85.1	45.8	22.5	203	9244.8	8369.0	47.5	8
ALLEGIANCE	9	12.5	14.5	88.9	47.2	25.7	171	7132.4	8209.6	53.5	9
KY03C-1195-10-1-5	10.5	13.5	15.5	81.3	39.3	30.8	174.5	7059.2	8094.7	53.4	10
KY05C-1617-17-17-3	9	13	15	86.4	43.8	26.7	160.5	6434.6	8093.1	55.7	11
KY06C-1003-139-8-3	10	12.5	14.5	83.8	47.0	30.6	200.5	9279.2	7690.0	45.3	12
IL07-20743	9.5	13.5	15.5	77.5	43.0	25.4	142	6361.2	7476.9	54.0	13
03207A1-7-3-1	9	12.5	14.5	78.7	45.7	31.5	156.5	6766.7	7456.2	52.4	14
KY04C-2006-41-1-1	9.5	13	15	87.6	47.3	20.3	167	7001.7	7428.8	51.5	15
IL01-11934	9.5	13	15	82.6	44.9	20.3	132.5	5734.0	7258.6	55.9	16
KY02C-1122-06	10.5	14	16	77.5	45.6	30.1	161	6493.4	7044.9	52.0	17
KY02C-3005-25	9	12	14	77.5	43.6	21.4	144	6534.3	6950.0	51.5	18
KY04C-2151-40	9.5	12	14	80.0	48.9	31.6	134	5572.8	6925.8	55.4	19
KY03C-1221-01	9	12.5	14.5	78.7	39.7	33.2	153	8956.8	6844.4	43.3	20
FOSTER	8.5	12	14	81.3	47.7	24.9	167	7828.8	6785.7	46.4	21
05222A1-1-2-1	9.5	13	15	83.8	42.1	31.4	158	6421.9	6685.8	51.0	22
04719A1-16-1-1-7	11	13.5	15.5	85.1	45.3	35.0	157	6239.1	6527.1	51.1	23
KY03C-2047-06	10	13	15	77.5	41.4	36.8	129.5	5906.4	6521.5	52.5	24
KY03C-1221-22	9	12.5	14.5	91.4	46.2	28.1	158.5	6627.0	6290.2	48.7	25
KY03C-1002-02	8.5	11.5	13.5	72.4	42.1	35.9	142	5338.6	6266.8	54.0	26
IL06-7550	9	11.5	13.5	80.0	43.1	28.6	106	4674.8	6167.1	56.9	27
KY03C-1192-37	10.5	13.5	15.5	71.1	42.1	33.5	140	6286.9	6033.6	49.0	28
04620A1-1-7-4	8.5	12	14	80.0	43.9	23.2	146.5	5994.1	5985.1	50.0	29
KY02C-2215-02	9	12.5	14.5	82.6	38.6	23.2	108	5770.1	5972.6	50.9	30
PEMBROKE	8.5	12	14	76.2	46.3	27.3	145.5	6254.7	5967.6	48.8	31
03633A1-69-2-5	8	12	14	83.8	43.2	19.4	76.5	4290.2	5964.7	58.2	32
KY04C-3006-33-14-3	9	11.5	13.5	72.4	50.3	29.7	114.5	4463.7	5846.7	56.7	33
KY03C-1237-15	8	11	13	83.8	40.7	26.6	104.5	4859.2	5662.8	53.8	34
IL07-6861	9	11.5	13.5	72.4	48.3	25.9	110	6126.9	5622.9	47.9	35
0537A1-3-12	9.5	12.5	14.5	77.5	46.4	28.1	139	5611.0	5618.0	50.0	36
KY02C-1058-03	9	13	15	85.1	45.1	26.8	143	4946.8	5310.6	51.8	37
KY03C-1237-32	9	12.5	14.5	78.7	47.0	22.7	96.5	4525.4	5271.5	53.8	38
KY04C-1128-38-1-5	9.5	12.5	14.5	81.3	46.3	31.8	119.5	5092.2	5173.4	50.4	39
KY93C-1238-17-1	8.5	11.5	13.5	72.4	48.6	30.1	111.5	3875.8	4273.4	52.4	40
$\bar{x}$	9.3	12.5	14.5	80.9	44.8	27.7	149.9	6512.2	6995.1	51.9	
CV	13.3	10.1	10.1	8.6	10.4	28.7	28.7	34.4	28.4	12.1	
SE	0.11	0.11	0.11	0.77	0.47	0.72	5.25	232.4	213.7	0.52	

Table 6.10. LSMEANS of nitrogen traits measured in 40 soft red winter wheat genotypes for the control treatment from the warming study Lexington, KY 2014. Mean ($\bar{x}$), coefficient of variance (CV), standard error (SE) from ANOVA represented below. Genotypes are ranked based on NUE, where NUE=Yield/N supply.

Name	% Grain protien	% N maturity	Vegetative N maturity (kg ha $^{-1}$)	Grain N (kg ha^{-1})	Total plant N (kg ha^{-1})	NHI (%)	NUtE (kg kg^{-1})	NUpE	NUE	Rank based on NUE
IL07-23420	10.58	0.41	39.4	165.0	204.4	80.7	48.1	1.43	68.1	1
KY05C-1105-42-20-1	10.98	0.41	34.8	167.1	201.9	82.7	47.4	1.41	66.5	2
IL07-20728	10.96	0.44	40.9	165.6	206.5	80.2	46.6	1.44	65.4	3
IL07-19334	11.59	0.40	29.6	172.5	202.1	85.3	46.0	1.41	64.3	4
IL07-21847	11.58	0.41	24.5	162.0	186.5	86.9	47.1	1.30	61.5	5
07290A1-12	10.95	0.37	26.6	153.0	179.6	85.2	49.1	1.25	61.0	6
KY03C-1237-01	11.32	0.39	31.5	160.6	192.1	83.6	46.1	1.34	60.4	7
05219A1-8-21-2-4	11.12	0.54	50.6	149.2	199.8	74.7	42.4	1.40	58.5	8
ALLEGIANCE	11.87	0.33	21.6	155.2	176.8	87.8	46.5	1.24	57.3	9
KY03C-1195-10-1-5	11.85	0.47	29.3	152.3	181.6	83.8	44.4	1.27	56.5	10
KY05C-1617-17-17-3	10.85	0.30	23.6	142.4	166.0	85.8	51.3	1.16	56.5	11
KY06C-1003-139-8-3	11.17	0.54	49.2	137.1	186.2	73.6	41.3	1.30	53.7	12
IL07-20743	11.66	0.49	30.9	139.4	170.3	81.9	43.9	1.19	52.2	13
03207A1-7-3-1	11.35	0.61	40.7	135.0	175.7	76.8	42.2	1.23	52.1	14
KY04C-2006-41-1-1	10.99	0.46	34.3	132.5	166.7	79.5	46.8	1.16	51.9	15
IL01-11934	11.54	0.46	25.1	133.7	158.7	84.2	45.9	1.11	50.7	16
KY02C-1122-06	11.66	0.50	33.1	134.5	167.6	80.3	43.8	1.17	49.2	17
KY02C-3005-25	10.51	0.52	33.4	116.5	149.9	77.7	46.3	1.05	48.5	18
KY04C-2151-40	11.75	0.49	31.3	132.7	164.0	80.9	45.4	1.15	48.4	19
KY03C-1221-01	12.44	0.68	56.8	136.6	193.3	70.6	35.3	1.35	47.8	20
FOSTER	11.88	0.48	34.2	128.3	162.6	78.9	41.3	1.14	47.4	21
05222A1-1-2-1	12.54	0.45	28.8	134.2	162.9	82.3	41.1	1.14	46.7	22
04719A1-16-1-1-7	11.97	0.47	26.8	124.6	151.4	82.3	43.2	1.06	45.6	23
KY03C-2047-06	12.50	0.61	36.3	129.5	165.8	78.1	39.2	1.16	45.5	24
KY03C-1221-22	11.18	0.45	31.1	112.2	143.3	78.3	44.3	1.00	43.9	25
KY03C-1002-02	12.29	0.59	30.4	122.6	153.0	80.1	41.1	1.07	43.8	26
IL06-7550	11.21	0.43	22.9	112.3	135.3	83.0	47.9	0.94	43.1	27
KY03C-1192-37	12.32	0.55	33.6	115.9	149.5	77.5	40.5	1.04	42.1	28
04620A1-1-7-4	11.28	0.59	36.0	108.0	144.0	75.0	41.8	1.01	41.8	29
KY02C-2215-02	11.68	0.67	39.4	111.7	151.1	73.9	41.1	1.06	41.7	30
PEMBROKE	12.09	0.59	34.9	113.3	148.2	76.5	40.1	1.03	41.7	31
03633A1-69-2-5	11.09	0.43	17.6	105.1	122.7	85.7	48.5	0.86	41.7	32
KY04C-3006-33-14-3	13.70	0.50	21.3	128.5	149.8	85.8	39.2	1.05	40.8	33
KY03C-1237-15	11.92	0.61	30.3	108.6	138.9	78.2	41.5	0.97	39.6	34
IL07-6861	12.32	0.52	30.7	109.5	140.2	78.1	40.3	0.98	39.3	35
0537A1-3-12	12.07	0.64	36.5	109.0	145.5	74.9	39.2	1.02	39.2	36
KY02C-1058-03	11.11	0.47	23.1	94.6	117.7	80.4	44.9	0.82	37.1	37
KY03C-1237-32	11.19	0.61	27.4	94.9	122.2	77.6	43.0	0.85	36.8	38
KY04C-1128-38-1-5	12.58	0.45	24.9	105.3	130.3	80.9	41.2	0.91	36.1	39
KY93C-1238-17-1	11.95	0.54	20.7	81.0	101.7	79.7	42.0	0.71	29.8	40
$\bar{x}$	11.6	0.49	31.9	129.8	161.6	80.2	43.7	1.13	48.9	
CV	6.49	26.8	41.6	28.5	28.9	8.2	11.4	28.9	28.4	
SE	0.10	0.01	1.31	3.55	4.09	0.64	0.53	0.03	1.5	

Table 6.11. LSMEANS of agronomic traits measured in 40 soft red winter wheat genotypes for the warmed treatment from the warming study Lexington, KY 2014. Mean ($\bar{X}$), coefficient of variance (CV), standard error (SE) from ANOVA represented below. Genotypes are ranked based on yield.

Name	Heading date (May)	Anthesis date (May)	Maturity date (June)	Height (cm)	CCI at anthesis	CCI at maturity	Plant number	Biomass (kg ha^{-1})	Yield (kg ha^{-1})	HI (%)	Rank based on yield
05219A1-8-21-2-4	5	8.5	6.5	73.66	46.0	16.2	215	9762.2	8839.3	47.5	1
IL06-7550	7	9.5	8	82.55	46.8	19.5	156	8633.6	8342.0	49.1	2
KY04C-2151-40	6	8.5	6.5	74.93	46.3	21.8	166.5	8054.7	8311.1	50.8	3
KY03C-1195-10-1-5	7	9	7	76.2	48.0	28.7	154.5	6723.5	8126.2	54.7	4
KY06C-1003-139-8-3	6.5	8.5	6.5	80.01	50.1	24.7	170	7794.1	7593.3	49.3	5
KY02C-3005-25	5.5	8	6	77.47	47.0	22.4	161.5	10479.8	7579.1	42.0	6
IL07-6861	5	9	7	76.2	45.0	16.2	148.5	8983.4	7534.4	45.6	7
KY03C-2047-06	7	9.5	7.5	77.47	43.0	23.1	151	7256.5	7406.0	50.5	8
KY03C-1221-01	5	8.5	6.5	77.47	49.4	20.9	169	7687.0	7283.9	48.7	9
04620A1-1-7-4	7	10.5	8.5	80.01	46.4	13.2	133.5	6480.2	7053.3	52.1	10
KY04C-1128-38-1-5	6	8	6	78.74	44.3	10.0	160	7498.9	6985.0	48.2	11
IL07-20728	6	8	6	80.01	46.3	12.9	138.5	6382.5	6952.6	52.1	12
KY04C-3006-33-14-3	5.5	8.5	6.5	83.82	37.5	10.8	92.5	4671.0	6852.1	59.5	13
KY02C-1122-06	6.5	8	6	73.66	46.5	19.0	162.5	6458.1	6747.1	51.1	14
05222A1-1-2-1	5	8	6	71.12	46.6	19.2	145	6078.3	6688.8	52.4	15
03633A1-69-2-5	6	9	7	77.47	49.1	16.7	133	5769.0	6379.4	52.5	16
03207A1-7-3-1	5.5	8.5	6.5	81.28	47.4	29.5	147.5	7003.9	6189.5	46.9	17
KY03C-1002-02	6	8.5	6.5	67.31	50.8	24.7	129.5	6682.9	5884.9	46.8	18
KY03C-1237-32	5	6	4.5	73.66	49.1	22.0	148.5	8672.5	5867.7	40.4	19
KY03C-1192-37	7	9	7	76.2	45.0	25.0	159.5	8459.8	5851.1	40.9	20
KY04C-2006-41-1-1	5	8	6	69.85	44.5	20.5	168	10670.9	5825.3	35.3	21
0537A1-3-12	7	9	7	77.47	43.4	12.5	142.5	6276.8	5823.6	48.1	22
IL01-11934	6	9	7	74.93	45.6	19.8	111	5398.3	5817.8	51.9	23
IL07-20743	5	8.5	6.5	83.82	45.5	17.3	143.5	6133.7	5690.9	48.1	24
KY03C-1237-01	6	8	6	71.12	51.3	14.1	155.5	8029.0	5617.4	41.2	25
IL07-23420	5.5	8	6	78.74	39.5	25.5	102	4457.3	5552.6	55.5	26
ALLEGIANCE	6	9	7	74.93	45.4	16.8	130.5	9008.2	5446.8	37.7	27
PEMBROKE	5	8	6	67.31	45.3	16.0	113.5	7400.9	5414.9	42.3	28
KY03C-1237-15	5	8	6	68.58	47.3	20.1	130.5	5539.7	5412.6	49.4	29
KY02C-1058-03	7	8	6	82.55	43.6	18.6	100	8873.1	5344.8	37.6	30
KY03C-1221-22	6	8	6	72.39	39.8	14.3	132	5666.9	5263.7	48.2	31
IL07-21847	6	8.5	6.5	76.2	44.6	14.2	153	7422.4	5228.3	41.3	32
KY05C-1617-17-17-3	6.5	9.5	7.5	69.85	47.9	21.2	109.5	6293.0	5173.7	45.1	33
07290A1-12	5.5	7.5	5.5	72.39	48.7	22.5	117	4077.0	4916.0	54.7	34
KY93C-1238-17-1	5.5	9	7	72.39	43.2	21.6	94.5	3785.7	4892.8	56.4	35
KY02C-2215-02	6	8.5	6.5	66.04	48.8	19.3	101	4265.0	4600.6	51.9	36
IL07-19334	5	8.5	6.5	78.74	40.1	17.3	117.5	5739.8	4356.5	43.1	37
04719A1-16-1-1-7	6	8.5	6.5	73.66	35.9	13.4	77	3429.7	4114.1	54.5	38
KY05C-1105-42-20-1	5	7.5	5.5	67.31	34.7	19.5	108.5	5042.5	3665.2	42.1	39
FOSTER	5.5	9	7	69.85	46.4	25.8	96.5	4371.4	3504.2	44.5	40
$\bar{X}$	5.9	8.5	6.5	75.2	45.3	19.1	136.1	6785.3	6103.2	47.8	
CV	13.3	10.1	10.1	8.6	10.4	28.7	28.7	34.4	28.4	12.1	
SE	0.11	0.11	0.11	0.76	0.60	0.75	4.5	288.0	204.203	0.90	

Table 6.12. LSMEANS of nitrogen traits measured for each of the 40 soft red winter wheat genotypes for the warmed treatment from the warming study Lexington, KY 2014. Mean ($\bar{x}$), coefficient of variance (CV), standard error (SE) from ANOVA represented below. Genotypes are ranked based on NUE. NUE is equivalent to yield. NUE=Yield/N supply.

NAME	% Grain protien	% N maturity	Vegetative N maturity (kg ha $^{-1}$)	Grain N (kg ha^{-1})	Total plant N (kg ha^{-1})	NHI (%)	NUtE (kg kg^{-1})	NUpE	NUE	Rank based on NUE
05219A1-8-21-2-4	11.38	0.89	86.9	160.3	247.2	64.9	35.7	1.73	61.7	1
IL06-7550	10.83	0.59	57.4	146.1	203.5	71.8	45.0	1.42	58.3	2
KY04C-2151-40	11.47	0.66	57.4	155.1	212.5	73.0	41.0	1.48	58.0	3
KY03C-1195-10-1-5	11.50	0.71	47.4	150.0	197.4	76.0	41.0	1.38	56.8	4
KY06C-1003-139-8-3	11.11	0.60	48.6	137.1	185.7	73.8	42.5	1.30	53.0	5
KY02C-3005-25	11.39	0.73	81.0	139.2	220.2	63.2	36.7	1.54	52.9	6
IL07-6861	11.58	0.57	50.8	139.8	190.7	73.3	39.5	1.33	52.6	7
KY03C-2047-06	11.29	0.52	37.7	133.4	171.1	78.0	43.3	1.20	51.7	8
KY03C-1221-01	10.46	0.60	45.8	122.7	168.5	72.8	43.4	1.18	50.9	9
04620A1-1-7-4	11.02	0.58	37.8	124.6	162.4	76.7	43.4	1.13	49.3	10
KY04C-1128-38-1-5	11.12	0.44	33.5	124.2	157.7	78.8	44.6	1.10	48.8	11
IL07-20728	10.95	0.69	47.2	122.6	169.8	72.2	42.8	1.19	48.6	12
KY04C-3006-33-14-3	11.01	0.46	21.6	120.3	141.9	84.8	48.3	0.99	47.9	13
KY02C-1122-06	10.98	0.60	39.5	120.1	159.6	75.2	42.9	1.11	47.1	14
05222A1-1-2-1	11.33	0.73	45.0	120.9	165.9	72.9	40.4	1.16	46.7	15
03633A1-69-2-5	10.91	0.66	35.7	111.8	147.6	75.8	43.1	1.03	44.6	16
03207A1-7-3-1	11.85	0.83	60.3	119.7	180.0	66.5	35.3	1.26	43.2	17
KY03C-1002-02	12.14	0.65	43.9	116.0	159.9	72.5	38.1	1.12	41.1	18
KY03C-1237-32	11.21	0.65	55.4	104.8	160.2	65.4	36.5	1.12	41.0	19
KY03C-1192-37	11.51	0.77	63.6	105.2	168.8	62.3	34.8	1.18	40.9	20
KY04C-2006-41-1-1	12.03	0.85	89.4	112.9	202.2	55.8	27.2	1.41	40.7	21
0537A1-3-12	11.15	0.74	49.3	104.7	154.0	68.0	40.8	1.08	40.7	22
IL01-11934	12.07	0.61	33.1	111.8	144.9	77.2	39.7	1.01	40.6	23
IL07-20743	12.08	0.60	35.8	110.1	145.9	75.5	39.4	1.02	39.7	24
KY03C-1237-01	11.50	0.77	62.5	105.2	167.7	62.7	34.2	1.17	39.2	25
IL07-23420	11.11	0.63	25.2	97.1	122.3	79.4	44.6	0.85	38.8	26
ALLEGIANCE	11.03	0.60	52.9	95.8	148.8	64.4	37.7	1.04	38.0	27
PEMBROKE	11.73	0.51	37.6	100.0	137.6	72.7	39.0	0.96	37.8	28
KY03C-1237-15	11.27	0.76	43.1	98.2	141.3	69.5	39.9	0.99	37.8	29
KY02C-1058-03	12.22	0.64	53.3	102.3	155.6	65.8	34.1	1.09	37.3	30
KY03C-1221-22	11.75	0.76	43.5	100.0	143.5	69.7	37.3	1.00	36.8	31
IL07-21847	11.35	0.69	50.2	96.2	146.4	65.7	35.4	1.02	36.5	32
KY05C-1617-17-17-3	10.93	0.69	41.7	90.3	131.9	68.4	39.2	0.92	36.1	33
07290A1-12	11.95	0.67	25.5	91.8	117.3	78.2	40.7	0.82	34.3	34
KY93C-1238-17-1	12.25	0.66	24.2	93.4	117.6	79.5	39.2	0.82	34.2	35
KY02C-2215-02	11.81	0.76	32.2	85.9	118.1	72.7	38.9	0.83	32.1	36
IL07-19334	11.48	0.64	39.0	81.3	120.3	67.6	39.8	0.84	30.4	37
04719A1-16-1-1-7	11.72	0.62	20.4	76.5	96.9	79.0	43.2	0.68	28.7	38
KY05C-1105-42-20-1	12.05	0.72	38.0	71.0	109.0	65.2	34.1	0.76	25.6	39
FOSTER	12.18	0.79	34.5	66.8	101.3	65.9	32.4	0.71	24.5	40
X	11.5	0.66	45.7	111.6	157.3	71.3	39.4	1.10	42.6	
CV	6.5	26.8	41.6	28.5	28.9	8.2	11.4	28.9	28.4	
SE	0.07	0.02	2.52	3.54	5.24	0.97	0.6	0.04	1.43	

Figure 6.1. Average monthly soil temperature in warmed and control treatments from temperature probes placed at a depth of 10 cm below the ground. 16 probes were placed in warmed treatment and 4 probes were placed in the control treatment. Temperature was collected every 15 minutes.

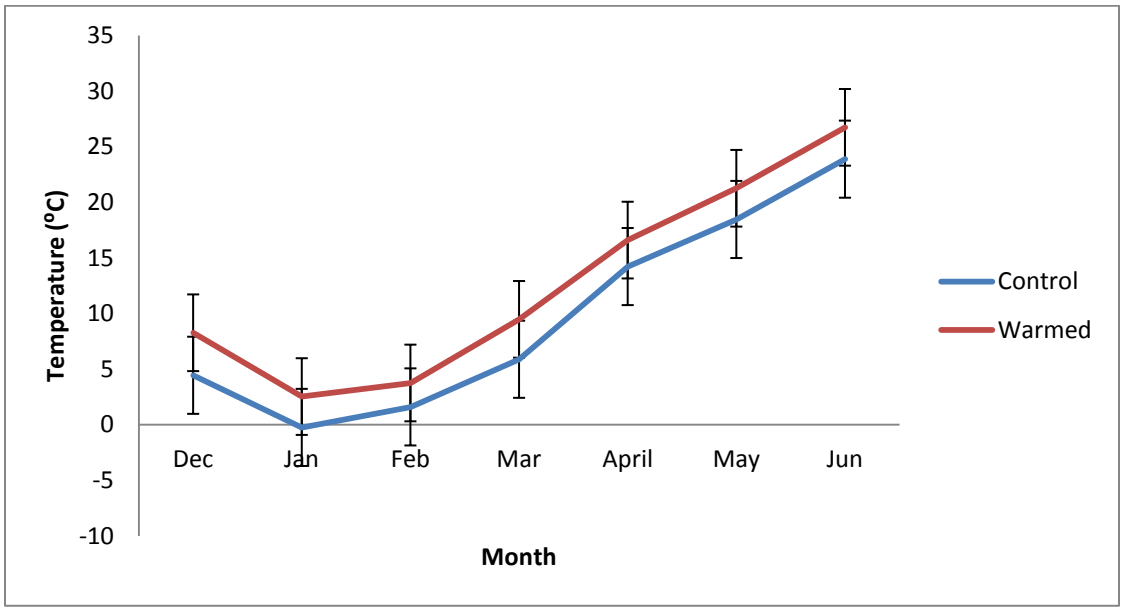


Figure 6.2. Average monthly air temperature in warmed and control treatments from temperature probes placed at a depth of 10 cm below the ground. 4 probes were placed in warmed treatment and 2 probes were placed in the control treatment. Temperature was collected every 15 minutes.

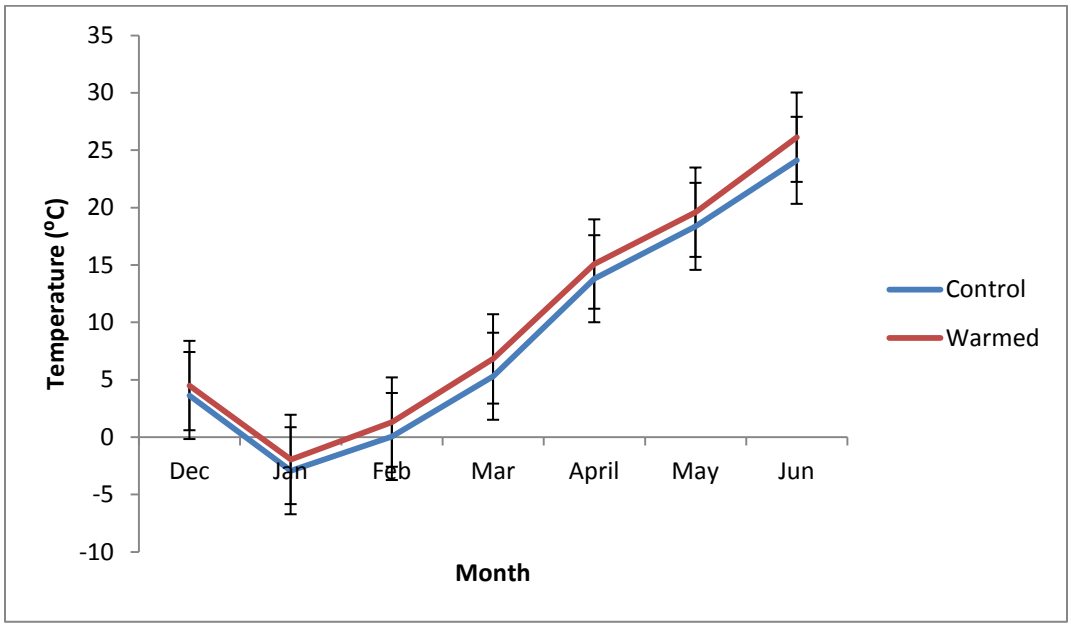


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Table A.4.1. Vegetative biomass at anthesis (Vba) (g m^{-2}), Vegetative biomass at maturity (Vbm) (g m^{-2}), and harvest index (HI) (%) LSMEANS for 8 soft red winter lines planted September (S), October (O), and November (N) under 0 kg N ha^{-1} (L), 101 kg N ha^{-1} (M), 168 kg N ha^{-1} (H) from the hill plot study Lexington, KY 2013.

		Vba			Vbm			HI		
Genotype		L	M	H	L	M	H	L	M	H
25R32	S	496.8	679.9	559.3	386.1	672.8	586.2	51.9	51.1	53.3
	O	56.8	90.8	217.2	45.4	123.5	220.0	59.0	58.6	57.1
	N	31.2	292.4	129.2	17.0	75.2	112.8	62.5	59.2	56.9
KY02C-1058-03	S	495.4	520.9	637.3	414.5	637.3	692.7	43.8	49.3	46.8
	O	52.5	140.5	160.4	78.1	168.9	139.1	51.3	53.9	51.0
	N	22.7	65.3	123.5	12.8	51.1	76.6	59.1	41.0	38.1
KY04C-1128-38-1-5	S	471.2	584.8	660.0	367.6	678.5	798.4	49.5	50.1	50.1
	O	58.2	124.9	164.7	48.3	144.8	184.5	57.5	56.4	51.9
	N	29.8	45.4	100.8	25.5	46.8	107.9	47.1	58.8	55.3
KY05C-1617-17-17-3	S	546.5	648.7	688.4	424.4	670.0	691.3	45.3	45.9	41.0
	O	75.2	143.4	130.6	45.4	171.7	262.6	55.6	53.3	52.1
	N	27.0	79.5	82.3	14.2	38.3	62.5	55.2	48.6	47.0
KY97C-1238-17-1	S	610.3	779.3	695.5	347.8	435.8	711.1	41.2	47.2	38.8
	O	97.9	160.4	176.0	73.8	198.7	123.5	54.0	51.6	53.5
	N	35.5	73.8	194.5	14.2	73.8	123.5	54.5	48.5	48.8
PEMBROKE	S	452.8	411.6	564.9	333.6	459.9	520.9	50.0	51.6	49.0
	O	90.8	156.1	183.1	75.2	150.5	203.0	51.8	54.1	55.2
	N	25.5	130.6	161.8	24.1	105.0	126.3	56.4	54.6	55.1
SHIRLEY	S	486.9	617.4	675.6	494.0	495.4	684.2	50.4	49.6	47.7
	O	62.5	117.8	177.4	85.2	212.9	210.1	56.5	57.9	55.3
	N	48.3	124.9	99.4	18.5	49.7	99.4	60.6	61.1	57.8
TRUMAN	S	660.0	694.1	797.7	502.5	635.9	748.0	47.2	50.1	47.1
	O	85.2	258.3	278.2	120.7	224.3	264.0	52.2	55.4	51.4
	N	45.4	127.7	141.9	25.5	137.7	156.1	59.1	50.8	52.0
$\bar{x}$		211.0	294.5	325.0	166.4	277.4	329.4	53.0	52.4	50.5
SE		47.5	50.4	50.8	36.8	48.0	53.5	1.1	1.0	1.11

Table A.4.2. % N in vegetative tissue at anthesis (Na), % N in vegetative tissue at maturity (Nm), and post-anthesis N uptake (PANU) (g m^{-2}) LSMEANS for 8 soft red winter lines planted September (S), October (O), and November (N) under 0 kg N ha^{-1} (L), 101 kg N ha^{-1} (M), 168 kg N ha^{-1} (H) from the hill plot study Lexington, KY 2013.

		Na			Nm			PANU		
Genotype		L	M	H	L	M	H	L	M	H
25R32	S	1.57	2.02	2.21	0.44	0.66	0.57	6.31	8.90	9.2
	O	1.59	2.14	2.35	0.65	0.60	0.86	0.59	1.30	3.3
	N	2.14	2.04	2.43	0.65	0.77	0.99	0.54	5.77	2.5
KY02C-1058-03	S	1.25	1.97	2.00	0.57	0.63	0.73	3.91	6.03	7.9
	O	1.86	2.37	2.65	0.79	0.58	1.04	0.39	2.97	-1.1
	N	2.47	2.33	2.68	0.81	0.92	1.37	0.45	1.10	3.6
KY04C-1128-38-1-5	S	1.64	1.60	2.13	0.44	0.52	0.64	6.13	6.05	8.5
	O	1.72	1.41	2.06	0.48	0.52	0.89	0.78	0.90	1.8
	N	2.31	2.75	2.68	0.86	0.86	0.84	0.45	0.85	1.9
KY05C-1617-17-17-3	S	1.74	2.63	1.81	0.84	0.65	0.92	7.82	12.25	6.0
	O	1.64	2.23	2.51	0.62	0.72	0.90	0.85	2.01	1.1
	N	1.94	2.51	2.78	0.87	1.01	1.06	0.38	1.49	1.6
KY97C-1238-17-1	S	1.45	1.65	1.77	0.52	0.64	0.89	6.94	9.98	5.9
	O	2.07	2.17	2.38	0.79	0.95	1.06	1.56	1.61	2.6
	N	2.85	2.85	2.77	0.79	0.93	1.15	0.88	1.54	3.8
PEMBROKE	S	1.55	1.67	1.81	0.60	0.57	0.52	5.19	4.15	7.8
	O	1.72	1.68	2.48	0.53	0.47	0.67	1.08	2.03	3.3
	N	1.79	2.12	1.98	0.74	0.89	0.85	0.37	1.74	2.1
SHIRLEY	S	1.57	1.87	2.06	0.47	0.82	0.95	5.21	7.48	7.3
	O	2.18	2.03	2.43	0.70	0.71	0.87	0.70	0.90	2.6
	N	2.22	2.06	2.64	0.84	0.99	1.10	0.94	1.69	1.1
TRUMAN	S	1.83	1.69	2.05	0.79	0.84	0.65	8.10	6.14	11.4
	O	1.70	1.93	1.97	0.89	0.85	0.60	0.45	1.70	4.3
	N	1.79	2.21	2.12	0.61	0.89	1.03	0.65	1.32	1.5
$\bar{x}$		1.9	2.1	2.3	0.68	0.75	0.88	2.5	3.7	4.2
SE		0.07	0.08	0.07	0.03	0.03	0.04	0.57	0.67	0.64

Table A.4.3. Vegetative N anthesis (Vna) (g m^{-2}), vegetative N maturity (Vnm) (g m^{-2}), and total plant N (TN) (g m^{-2}) LSMEANS for 8 soft red winter lines planted September (S), October (O), and November (N) under 0 kg N ha^{-1} (L), 101 kg N ha^{-1} (M), 168 kg N ha^{-1} (H) from the hill plot study Lexington, KY 2013.

		Vna			Vnm			TN		
Genotype		L	M	H	L	M	H	L	M	H
25R32	S	7.6	13.5	12.6	1.7	4.6	3.4	10.3	22.5	21.7
	O	0.9	2.1	5.1	0.3	0.8	1.8	1.8	5.1	9.6
	N	0.6	6.4	3.2	0.1	0.6	1.1	0.7	3.3	5.3
KY02C-1058-03	S	6.4	9.9	13.1	2.3	3.9	5.2	9.4	19.7	22.0
	O	1.0	3.8	2.3	0.6	0.8	1.6	2.5	5.7	5.5
	N	0.6	1.6	3.1	0.1	0.5	0.7	0.5	1.4	2.7
KY04C-1128-38-1-5	S	7.8	9.5	14.0	1.6	3.4	5.1	8.9	18.3	26.0
	O	1.0	1.7	3.4	0.2	0.8	1.6	1.8	5.1	6.9
	N	0.7	1.3	2.7	0.2	0.4	0.8	0.9	2.0	4.4
KY05C-1617-17-17-3	S	9.7	16.7	12.4	3.9	4.4	6.4	11.9	22.9	19.9
	O	1.1	3.4	3.3	0.3	1.4	2.2	1.6	6.4	9.7
	N	0.5	2.1	2.3	0.1	0.6	0.6	0.6	1.9	2.1
KY97C-1238-17-1	S	8.9	12.8	12.1	1.9	2.8	6.2	7.4	11.9	18.2
	O	2.2	3.3	3.9	0.6	1.8	1.3	2.5	7.1	5.1
	N	1.0	2.1	5.2	0.1	0.5	1.4	0.5	2.2	4.5
PEMBROKE	S	7.0	6.9	10.3	1.8	2.8	2.5	9.3	15.5	16.0
	O	1.5	2.7	4.6	0.4	0.7	1.3	2.4	4.9	7.9
	N	0.5	2.8	3.1	0.2	1.0	1.0	0.9	4.2	5.0
SHIRLEY	S	7.6	11.5	13.8	2.4	4.0	6.5	15.1	14.8	21.8
	O	1.4	2.4	4.4	0.7	1.5	1.8	3.1	8.5	8.7
	N	1.1	2.2	2.1	0.1	0.5	1.1	0.8	2.4	4.7
TRUMAN	S	12.2	11.5	16.3	4.1	5.4	4.9	16.7	22.6	22.9
	O	1.5	5.1	5.9	1.1	1.7	1.6	4.3	7.8	9.5
	N	0.8	2.8	3.0	0.2	1.5	1.5	1.1	5.4	6.0
$\bar{x}$		3.5	5.7	6.8	1.0	1.9	2.6	4.8	9.2	11.1
SE		0.76	0.94	0.97	0.24	0.32	0.40	1.0	1.5	1.6

Table A.5.1. Heading date (HD) (May), anthesis date (AD) (May), and height LSMEANS for the 56 soft red winter lines grown at Princeton (PRN) and Lexington (LEX), KY under 0 kg ha^{-1} (L) and kg ha^{-1} (H) N. Mean ($\bar{x}$), standard error (SE).

Genotype	HD				AD				Height (cm)			
	PRN		LEX		PRN		LEX		PRN		LEX	
	L	H	L	H	L	H	L	H	L	H	L	H
011007A1-14-16-50	6	9	13.5	13	10	13	15	15	59.7	76.2	69.9	61.0
03207A1-7-3-1	6	8	14	14	9.5	12	15	15	54.6	77.5	59.7	58.4
03633A1-69-2-5	6.5	8.5	13.5	13	10.5	12.5	14.5	14.5	62.2	73.7	71.1	76.2
04620A1-1-7-4	6.5	8	13.5	13	10.5	12	14	14	69.9	86.4	64.8	80.0
04719A1-16-1-1-7	7	8.5	13	14	10.5	12.5	14	16	69.9	77.5	61.0	68.6
05219A1-8-21-2-4	5.5	8	13.5	14	9.5	12	15.5	16	61.0	82.6	67.3	74.9
05222A1-1-2-1	6	8	13.5	13	10	12	14	14	62.2	80.0	67.3	67.3
0537A1-3-12	6	8.5	13.5	14.5	10	12.5	15.5	16.5	69.9	85.1	64.8	81.3
07290A1-12	6	8	13.5	13.5	9.5	12	15	15.5	61.0	83.8	58.4	76.2
ALLEGIANCE	6	8	13	14	10	12	14	15.5	67.3	87.6	77.5	83.8
FOSTER	6	8	14	15	10	12	15	17	73.7	87.6	73.7	76.2
IL01-11934	6	8	13	13.5	10	12	14	15.5	76.2	87.6	71.1	74.9
IL06-13072	5.5	8	13	13	10	12	14	14.5	72.4	87.6	68.6	83.8
IL06-7550	6.5	8	13	14	10	12	15.5	16.5	71.1	88.9	72.4	78.7
IL07-19334	6	8	14	14	10	12	15.5	16	67.3	91.4	68.6	77.5
IL07-20728	7	8.5	13	14	10	12.5	14	15	68.6	91.4	64.8	82.6
IL07-20743	6.5	8	13	14	10	12	14	15	78.7	90.2	74.9	81.3
IL07-21847	6.5	8.5	13.5	13.5	10.5	13	14.5	15	71.1	92.7	72.4	73.7
IL07-23420	6.5	8	13.5	13	10	12	14.5	14.5	66.0	86.4	72.4	77.5
IL07-6861	6	8.5	13	14	10	12.5	14	15	78.7	82.6	73.7	78.7
IL08-34020	7	8	13	13	10.5	12	14	14.5	66.0	87.6	72.4	69.9
IL99-26442	6	8	13	14	10	12.5	16	16.5	81.3	90.2	81.3	87.6
KY02C-1058-03	6	8.5	13	15	10	12.5	16	17.5	67.3	85.1	69.9	80.0
KY02C-1076-07	6	8	14	15	10	12	15	17	67.3	83.8	68.6	76.2
KY02C-1121-11	6	8	13	14	10	12	14	16	58.4	88.9	64.8	77.5
KY02C-1121-75	6.5	8	13	13.5	10	12	14	15	68.6	83.8	64.8	78.7
KY02C-1122-06	6	8.5	14	13.5	10	12	14	15	72.4	86.4	68.6	78.7
KY02C-2215-02	7.5	8.5	13	14	11	12.5	15	16	67.3	88.9	69.9	80.0
KY02C-3004-07	6.5	8.5	13	14	10.5	13	15	16	68.6	87.6	63.5	77.5
KY02C-3005-25	6	9	13	14	10	12.5	14	15	73.7	85.1	71.1	77.5
KY03C-1002-02	6	8	13	14.5	10	12.5	15	16.5	63.5	85.1	69.9	83.8
KY03C-1192-37	6	8	14	15	10	12	15	17	69.9	87.6	63.5	73.7
KY03C-1195-10-1-5	6	9	13	14	10	12.5	14	15	69.9	78.7	68.6	77.5
KY03C-1221-01	6	8	13	14	10	12.5	15.5	17.5	72.4	88.9	63.5	72.4
KY03C-1221-06	6	9.5	13	14	10	13.5	16	16	68.6	77.5	64.8	73.7
KY03C-1221-22	6	9	13	14	10	13	15.5	17	63.5	85.1	69.9	72.4
KY03C-1237-01	6	8.5	13	14	10.5	12	14	16	68.6	87.6	62.2	74.9
KY03C-1237-15	5.5	8	14	15	9.5	13	15.5	17	66.0	87.6	71.1	77.5
KY03C-1237-32	6.5	8	13	14	10.5	12.5	15	17	66.0	87.6	57.2	74.9
KY03C-2047-02	6	8	13	14	10	12	14	14.5	69.9	80.0	59.7	74.9
KY03C-2047-06	6	8.5	13	13.5	10.5	12.5	14	14.5	66.0	87.6	61.0	74.9
KY03C-2049-02	6	8	13	14	10	12	16	17.5	61.0	87.6	69.9	76.2
KY03C-2314-08	6	8.5	13	14	10	13	14	14.5	62.2	83.8	67.3	81.3
KY03C-2399-02	6.5	8	13	14	10.5	12	14	14.5	62.2	82.6	59.7	71.1
KY04C-1128-38-1-5	6	8	13.5	14.5	10	12	15.5	16.5	72.4	87.6	81.3	78.7
KY04C-2006-41-1-1	6	8.5	13	14.5	10	12.5	15.5	17	59.7	90.2	72.4	74.9
KY04C-2151-40	6	8.5	13	14	10	12.5	15	16.5	73.7	92.7	69.9	80.0
KY04C-2151-41	6	8	13	13	10	13	14	14	71.1	81.3	62.2	73.7
KY04C-3006-33-14-3	6.5	8.5	13.5	13.5	10	12.5	14.5	15	68.6	82.6	69.9	78.7
KY05C-1007-2-12-5	5.5	8	13	15.5	9.5	12	16	17.5	72.4	88.9	72.4	83.8
KY05C-1105-42-20-1	6	8	13	13	10	12	14	14	67.3	85.1	71.1	80.0
KY05C-1381-77-7-5	6	9	13	14	9.5	13	14	16	71.1	91.4	76.2	80.0
KY05C-1617-17-17-3	6	8.5	13.50	13.50	10	12.5	14.5	14	67.3	90.2	77.5	64.8
KY06C-1003-139-8-3	6.5	8.5	13	14	10.5	12.5	15	15.5	66.0	88.9	68.6	78.7
KY93C-1238-17-1	6.5	8.5	13	14	10.5	12.5	14	15.5	72.4	88.9	76.2	82.6
PEMBROKE	6	8	13	14.5	9	12.5	15	16.5	71.1	86.4	69.9	80.0
$\bar{x}$	6.2	8.3	13.2	13.9	10.1	12.4	14.7	15.7	68.1	85.8	68.6	76.6
SE	0.1	0.0	0.0	0.1	0.05	0.05	0.09	0.14	0.70	0.58	0.73	0.72

Table A.5.2. Vegetative biomass at anthesis (Vba), Vegetative biomass at maturity (Vbm), and harvest index (HI) LSMEANS for 56 soft red winter lines grown at Princeton (PRN) and Lexington (LEX), KY under 0 kg N ha^{-1} (L) and 112 kg N ha^{-1} (H). Mean ($\bar{x}$), standard error (SE).

Genotype	Vba (kg ha^{-1})				Vbm (kg ha^{-1})				HI (%)			
	PRN		LEX		PRN		LEX		PRN		LEX	
	L	H	L	H	L	H	L	H	L	H	L	H
011007A1-14-16-50	2945.26	4344.9	3248.35	3482.3	1515.4	2385.8	1604.9	2603.4	46.0	64.7	56.1	60.7
03207A1-7-3-1	4629.25	3968.6	3906.06	3974.4	1815.8	2828.8	1964.4	3027.5	48.1	58.8	53.0	56.7
03633A1-69-2-5	3376.07	4308.1	3228.35	3823.8	2112.9	3547.0	1324.8	2660.8	57.5	54.4	60.7	60.5
04620A1-1-7-4	3423.28	4056.1	3664.19	3678.9	1999.9	2575.0	2265.6	2848.6	53.6	64.4	49.6	59.9
04719A1-16-1-1-7	3680.34	4438.6	3187.68	3800.4	2434.6	2255.0	1585.0	2248.8	53.7	65.6	56.8	63.5
05219A1-8-21-2-4	2201.71	4218	3602.46	3814.8	1565.7	2589.9	1848.1	2955.3	55.8	63.0	52.2	61.3
05222A1-1-2-1	4757.65	4395.3	3692.77	3573.8	1813.1	2899.4	2291.9	3010.5	42.9	61.0	49.4	58.1
0537A1-3-12	3962.61	4068	3805.82	3143.6	1837.7	3240.9	2223.0	1814.0	53.8	61.3	55.2	72.8
07290A1-12	3105.33	4277.1	3591.85	3299.8	1787.9	2857.2	1996.2	2894.9	54.7	61.8	56.3	58.5
ALLEGIANCE	3332.39	4344.9	3898.55	3588.6	2343.7	3118.0	2946.8	2627.2	49.3	57.2	47.2	65.2
FOSTER	3225.33	4147.2	3609.81	3278	2184.9	3091.0	1982.7	2780.1	45.2	59.5	58.1	63.3
IL01-11934	4893.64	4523.6	2760.82	3624.2	2574.2	2834.4	2056.1	2924.0	48.5	62.7	59.6	65.6
IL06-13072	4271.74	4355	3404.5	3894.9	1578.3	3214.8	1870.2	3174.9	56.1	60.8	56.7	61.0
IL06-7550	4175.62	4383.3	3892.42	3721.5	2303.4	2837.3	2453.9	2986.4	48.0	64.1	51.6	63.2
IL07-19334	3452.9	4677.4	2476.33	3726.9	1816.0	3050.6	2494.6	3131.2	52.3	62.5	50.7	55.5
IL07-20728	3619.33	3597.7	4423	3546	2679.2	3078.4	1750.9	2694.5	53.1	61.6	59.2	69.8
IL07-20743	3606.55	4389.7	3625.54	4153.6	2034.2	2710.8	1767.8	2915.6	48.3	64.0	62.2	61.7
IL07-21847	3203.32	4192.8	3295.04	3207	1973.5	3026.9	2206.4	2755.7	48.7	58.5	56.5	63.7
IL07-23420	3393.55	4667.9	3553.22	3325.6	1854.5	3087.1	2618.4	2835.3	51.1	60.2	54.4	64.5
IL07-6861	4178.59	3716.1	3908.99	3722.8	2905.2	3726.5	1322.1	3142.3	59.4	55.4	66.6	59.5
IL08-34020	3491.58	4851	3649.23	3680.3	2331.3	3084.4	2428.2	2935.1	52.1	60.4	46.6	54.0
IL99-26442	2575.39	4221.1	3929.87	3983	2105.8	3098.5	2740.9	2975.4	55.4	57.4	53.4	60.6
KY02C-1058-03	3879.61	4364.2	3708.48	3803.7	2132.1	3346.5	2301.9	3048.7	55.0	56.0	50.6	59.0
KY02C-1076-07	4177.56	4657.4	3327.79	3820.1	1895.9	3122.5	2041.9	2999.9	42.9	59.4	57.9	60.6
KY02C-1121-11	4211.52	4908.2	3820.28	3724.6	2024.6	3232.6	2226.2	3089.7	56.0	56.5	57.9	64.9
KY02C-1121-75	4016.13	3901.5	2864.48	3674.9	2684.3	2983.5	2131.0	2893.7	52.8	59.9	61.0	66.2
KY02C-1122-06	3426.25	4200.9	3553.54	3544.5	2253.2	3003.8	1168.4	3238.7	56.8	61.7	67.3	58.3
KY02C-2215-02	3619.71	4174.1	2930.48	3512.8	2029.8	3528.3	1965.9	2485.3	52.3	58.3	58.3	67.2
KY02C-3004-07	4505.97	4709.1	4040.5	3367.5	2023.8	3262.7	2073.8	2725.4	56.8	56.1	56.8	66.6
KY02C-3005-25	4278.89	3975.2	3347.56	3771.6	2007.8	3040.5	1428.1	2731.5	50.9	60.7	65.9	61.7
KY03C-1002-02	4207.65	4407.9	3786.29	3756.2	2103.1	2813.5	2180.8	3083.0	54.5	64.9	54.5	62.5
KY03C-1192-37	4791.56	4693.5	4439.42	3805.7	2485.5	3188.5	1694.3	2929.2	57.5	59.8	62.1	60.3
KY03C-1195-10-1-5	4070.65	4745.6	3500.56	3299.8	2404.8	3539.0	2424.4	3204.4	50.0	55.3	54.4	58.7
KY03C-1221-01	3489.3	4739.4	3383.5	3499.1	1996.0	3521.3	1743.2	2824.8	44.9	55.2	54.7	57.4
KY03C-1221-06	4148.4	4652.1	2818.58	3978.7	2404.9	2717.0	2274.0	2667.3	54.5	60.8	54.2	58.9
KY03C-1221-22	3210.47	4620.5	3619.16	3600.3	1856.0	2777.4	2855.1	3224.4	52.6	61.7	43.4	55.7
KY03C-1237-01	4035.67	3987.7	3301.61	3459.6	2192.1	3052.0	2394.4	2387.6	60.5	59.5	55.2	70.9
KY03C-1237-15	3325.11	4372.2	3534.19	3645	2273.4	2149.9	2737.2	2536.5	48.0	66.3	47.5	64.7
KY03C-1237-32	3632.48	5125	3912.32	3992.9	2378.5	2877.9	2416.4	2860.0	55.6	60.3	52.5	60.8
KY03C-2047-02	3273.76	4627.3	3238.11	3381.7	2672.1	3508.6	1688.0	2818.3	57.2	57.1	60.8	62.8
KY03C-2047-06	4343.87	4455.8	3731.74	3693.9	2166.1	3235.1	2335.1	2679.3	62.0	59.8	54.2	62.9
KY03C-2049-02	3968.85	4328	3435.25	3548.4	2328.6	3325.7	1613.1	2986.9	52.0	54.6	66.2	57.9
KY03C-2314-08	3908.57	4252.5	3678.52	3776.4	2322.7	2521.8	1959.5	2583.0	55.1	65.0	61.1	70.5
KY03C-2399-02	3301.19	4497.2	3298.85	3499.5	1932.0	2673.6	2858.5	2265.6	53.0	64.5	47.3	65.3
KY04C-1128-38-1-5	4605.75	4950.3	3492.04	3756.4	1606.7	3308.5	2935.3	2103.4	53.8	58.2	48.9	66.2
KY04C-2006-41-1-1	3668.47	4349.8	3644.4	3693.9	1736.9	2876.2	2008.1	2565.0	49.6	63.3	61.9	66.3
KY04C-2151-40	4429.45	4446.3	2887.42	3477.5	1801.6	2984.8	2301.7	2561.7	49.8	60.4	52.3	65.2
KY04C-2151-41	4868.09	4873.6	3530.58	3688.2	3103.5	2781.9	1992.1	3081.5	51.9	60.2	55.0	60.7
KY04C-3006-33-14-3	4300.64	4587.4	3904.65	3555	1701.5	2822.1	2638.5	2725.0	56.5	57.3	52.0	65.3
KY05C-1007-2-12-5	4047.99	4375.8	3499.8	3659.2	2428.0	2653.4	2445.5	3012.6	51.6	64.4	54.0	64.8
KY05C-1105-42-20-1	4169.32	4387.2	2478.77	3732.6	2867.7	3180.7	1620.5	2515.4	54.2	57.0	60.6	68.7
KY05C-1381-77-7-5	3906.17	4204.7	2855.96	3768.9	1790.5	2324.5	3076.1	2691.6	57.8	64.8	47.3	65.4
KY05C-1617-17-17-3	3842.41	4880.3	3083.42	3781.1	2992.7	3278.3	3177.4	2823.5	49.5	58.6	48.8	61.6
KY06C-1003-139-8-3	4248.77	4171.8	2920.43	3538.5	2704.5	2998.2	2899.8	2697.7	58.7	61.6	50.5	66.0
KY93C-1238-17-1	3653.53	4377.1	3729.77	3068.3	1852.4	3075.0	2230.0	2595.3	57.3	62.5	60.2	67.3
PEMBROKE	3499.42	4579.8	3722.67	3663.7	2146.9	3068.1	1615.2	2216.6	54.6	60.5	65.4	69.9
$\bar{x}$	3831.5	4405.7	3488.9	3635.4	2158.3	2998.4	2164.2	2782.0	52.9	60.4	55.6	62.9
SE	75.2	41.0	55.5	29.5	50.2	44.68	62.96	39.34	0.57	0.42	0.75	0.56

Table A.5.3. Percent grain protein (%Gp), N concentration at anthesis in vegetative tissue (%Na), and N concentration at maturity in vegetative tissue (%Nm) LSMEANS for 56 soft red winter lines grown at Princeton (PRN) and Lexington (LEX), KY under 0 kg N ha^{-1} (L) and 112 kg N ha^{-1} (H) N. Mean ($\bar{x}$), standard error (SE).

Genotype	%Gp				%Na				%Nm			
	PRN		LEX		PRN		LEX		PRN		LEX	
	L	H	L	H	L	H	L	H	L	H	L	H
011007A1-14-16-50	10.3	10.1	10.0	10.4	1.18	1.32	1.28	1.79	0.39	0.35	0.79	0.76
03207A1-7-3-1	10.1	10.5	10.4	11.8	1.17	1.34	1.70	2.02	0.43	0.37	0.81	0.42
03633A1-69-2-5	10.0	10.1	10.4	9.2	1.42	1.16	1.54	1.85	0.29	0.47	0.73	0.57
04620A1-1-7-4	10.4	9.5	10.0	9.8	1.12	1.32	1.50	2.04	0.40	0.30	0.58	0.45
04719A1-16-1-1-7	10.4	9.9	10.0	10.5	1.16	1.36	1.70	1.68	0.34	0.36	0.70	0.61
05219A1-8-21-2-4	10.0	10.5	10.3	11.2	1.24	1.21	1.39	1.91	0.48	0.31	0.66	0.64
05222A1-1-2-1	10.2	9.8	10.2	10.6	1.22	1.30	1.49	1.55	0.47	0.44	0.60	0.57
0537A1-3-12	10.3	9.7	9.4	10.1	1.31	1.21	1.53	1.63	0.38	0.30	0.67	0.56
07290A1-12	9.8	9.8	9.5	10.8	1.28	1.36	1.52	2.12	0.38	0.26	0.65	0.46
ALLEGIANCE	9.6	10.2	9.2	9.5	1.30	1.29	1.52	1.89	0.29	0.25	0.60	0.47
FOSTER	10.0	9.6	9.3	10.8	1.27	1.29	1.81	1.70	0.31	0.32	0.66	0.48
IL01-11934	9.6	11.1	8.9	9.3	1.14	1.25	1.51	1.76	0.27	0.25	0.56	0.54
IL06-13072	9.7	9.4	9.6	9.2	1.21	1.34	1.46	1.87	0.42	0.21	0.66	0.39
IL06-7550	10.2	9.2	8.9	9.6	1.22	1.27	1.76	1.83	0.25	0.28	0.59	0.48
IL07-19334	9.6	9.2	9.0	8.7	1.20	1.24	1.55	1.83	0.34	0.25	0.67	0.49
IL07-20728	9.8	9.7	9.4	9.4	1.29	1.24	1.41	1.63	0.27	0.23	0.75	0.50
IL07-20743	9.7	9.8	9.4	9.4	1.25	1.14	1.49	1.45	0.41	0.20	0.71	0.47
IL07-21847	9.8	9.7	9.0	9.2	1.27	1.33	1.46	2.04	0.39	0.37	0.71	0.49
IL07-23420	9.9	9.5	9.2	10.0	1.26	1.25	1.50	1.79	0.37	0.31	0.62	0.52
IL07-6861	9.5	9.6	9.5	10.7	1.24	1.34	1.70	1.80	0.20	0.25	0.77	0.63
IL08-34020	9.5	9.5	9.1	9.4	1.25	1.15	1.38	1.76	0.24	0.33	0.57	0.53
IL99-26442	10.2	9.5	9.5	9.8	1.30	1.36	1.50	1.52	0.41	0.21	0.42	0.42
KY02C-1058-03	9.6	9.8	10.1	10.2	1.25	1.30	1.28	1.79	0.33	0.21	0.74	0.51
KY02C-1076-07	9.8	9.4	9.1	9.5	1.14	1.24	1.44	1.73	0.43	0.27	0.62	0.46
KY02C-1121-11	10.1	9.8	9.1	10.1	1.22	1.23	1.57	1.91	0.48	0.31	0.51	0.62
KY02C-1121-75	9.7	9.3	8.9	9.2	1.19	1.22	1.59	1.82	0.33	0.25	0.77	0.38
KY02C-1122-06	9.9	9.5	9.4	9.7	1.22	1.18	1.64	1.90	0.34	0.03	0.63	0.41
KY02C-2215-02	9.8	9.5	10.1	9.6	1.37	1.24	1.40	1.77	0.34	0.24	0.66	0.46
KY02C-3004-07	9.7	9.7	10.2	10.3	1.27	1.28	1.64	1.70	0.36	0.28	0.80	0.69
KY02C-3005-25	9.6	9.6	9.3	9.8	1.23	1.18	1.83	1.75	0.36	0.26	0.62	0.49
KY03C-1002-02	10.1	9.8	9.4	10.5	1.18	1.31	1.47	1.62	0.39	0.22	0.65	0.52
KY03C-1192-37	10.2	9.6	9.4	9.5	1.09	1.24	1.55	1.98	0.21	0.34	0.70	0.54
KY03C-1195-10-1-5	9.7	10.0	9.6	9.6	1.37	1.26	1.65	1.80	0.32	0.21	0.63	0.31
KY03C-1221-01	10.3	9.9	9.9	10.9	1.41	1.46	1.62	1.92	0.35	0.34	0.56	0.56
KY03C-1221-06	10.3	10.2	9.6	10.0	1.22	1.24	1.70	1.75	0.33	0.38	0.77	0.60
KY03C-1221-22	9.6	9.8	9.3	9.4	1.28	1.21	1.53	1.88	0.41	0.28	0.56	0.54
KY03C-1237-01	9.5	9.7	10.1	10.1	1.28	1.22	1.64	1.74	0.28	0.27	0.68	0.66
KY03C-1237-15	9.7	9.5	9.3	9.3	1.22	1.22	1.42	1.78	0.45	0.41	0.78	0.63
KY03C-1237-32	10.0	9.5	10.2	9.9	1.30	1.18	1.53	1.72	0.37	0.17	0.64	0.40
KY03C-2047-02	9.8	10.0	9.1	9.6	1.15	1.27	1.40	1.60	0.35	0.33	0.69	0.46
KY03C-2047-06	9.8	9.7	9.4	9.3	1.18	1.25	1.55	1.75	0.35	0.35	0.61	0.35
KY03C-2049-02	10.2	9.8	9.5	9.6	1.26	1.26	1.74	1.89	0.45	0.15	0.71	0.49
KY03C-2314-08	9.2	9.3	8.9	9.4	1.24	1.41	1.57	1.72	0.39	0.29	0.65	0.57
KY03C-2399-02	10.1	9.5	9.4	9.2	1.38	1.35	1.56	1.92	0.37	0.16	0.70	0.44
KY04C-1128-38-1-5	9.9	9.8	9.5	9.4	1.16	1.17	1.64	2.33	0.35	0.32	0.62	0.44
KY04C-2006-41-1-1	9.7	9.8	9.0	9.2	1.27	1.23	1.80	1.80	0.46	0.37	0.74	0.43
KY04C-2151-40	10.5	10.1	10.1	10.4	1.17	1.29	1.66	1.92	0.41	0.28	0.72	0.53
KY04C-2151-41	10.9	10.0	10.0	10.0	1.16	1.24	1.63	1.83	0.31	0.29	0.68	0.54
KY04C-3006-33-14-3	10.3	10.1	10.2	9.7	1.34	1.16	1.52	1.73	0.30	0.20	0.70	0.42
KY05C-1007-2-12-5	9.9	9.5	9.3	9.4	1.27	1.25	1.53	1.84	0.37	0.20	0.55	0.41
KY05C-1105-42-20-1	10.7	9.7	9.5	9.5	1.20	1.21	1.39	2.10	0.31	0.30	0.61	0.46
KY05C-1381-77-7-5	9.5	10.0	8.8	10.2	1.19	1.19	1.76	2.02	0.45	0.32	0.52	0.30
KY05C-1617-17-17-3	9.5	9.5	9.2	9.4	1.21	1.29	1.53	1.94	0.32	0.29	0.51	0.33
KY06C-1003-139-8-3	9.4	9.9	8.7	9.3	1.35	1.24	1.42	1.92	0.38	0.26	0.64	0.53
KY93C-1238-17-1	9.6	9.3	9.0	9.2	1.26	1.29	1.95	2.28	0.34	0.41	0.69	0.55
PEMBROKE	10.5	9.4	10.3	9.9	1.21	1.22	1.70	1.89	0.41	0.22	0.60	0.41
$\bar{x}$	9.92	9.74	9.52	9.81	1.24	1.26	1.56	1.83	0.36	0.28	0.65	0.50
SE	0.05	0.05	0.06	0.08	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01

Table A.5.4. N content in vegetative tissue at anthesis, post-anthesis N uptake (PANU), normalized difference vegetative index (NDVI) LSMEANS for 56 soft red winter lines grown at Princeton (PRN) and Lexington (LEX), KY under 0 kg N ha^{-1} (L) and 112 kg N ha^{-1} (H). Mean ($\bar{x}$), standard error (SE).

Genotype	Nva (kg ha^{-1})				PANU (kg ha^{-1})				NDVI			
	PRN		LEX		PRN		LEX		PRN		LEX	
	L	H	L	H	L	H	L	H	L	H	L	H
011007A1-14-16-50	34.6	57.4	41.6	62.2	4.9	21.9	3.8	24.7	0.39	0.68	0.38	0.62
03207A1-7-3-1	53.9	53.0	66.4	80.1	-12.2	25.4	-13.5	8.1	0.37	0.62	0.51	0.65
03633A1-69-2-5	47.8	50.0	49.7	70.8	-4.7	35.1	-6.0	3.0	0.43	0.60	0.47	0.60
04620A1-1-7-4	38.2	53.5	55.0	74.9	9.3	24.9	0.8	4.6	0.44	0.70	0.58	0.69
04719A1-16-1-1-7	42.5	60.1	54.0	63.7	3.6	16.3	-9.9	15.8	0.39	0.60	0.38	0.58
05219A1-8-21-2-4	27.3	50.8	49.9	72.9	16.7	30.7	-0.9	29.8	0.42	0.69	0.47	0.80
05222A1-1-2-1	57.8	57.1	55.0	55.4	-14.1	25.9	-3.1	32.2	0.40	0.72	0.47	0.70
0537A1-3-12	51.9	49.2	58.2	51.2	-6.4	40.3	-2.4	43.3	0.45	0.71	0.47	0.69
07290A1-12	39.6	58.2	54.6	69.8	-0.7	21.0	-2.7	14.5	0.43	0.68	0.45	0.66
ALLEGIANCE	43.3	56.0	59.1	67.8	-4.0	19.4	-2.5	19.3	0.44	0.66	0.48	0.76
FOSTER	40.8	53.3	65.3	55.7	2.2	25.7	-11.3	40.2	0.50	0.74	0.41	0.75
IL01-11934	55.8	56.3	41.6	63.8	-8.3	34.1	15.7	34.4	0.46	0.69	0.53	0.75
IL06-13072	51.7	58.4	49.6	72.6	-5.2	23.2	0.4	12.5	0.42	0.69	0.38	0.70
IL06-7550	50.7	55.7	68.3	67.9	-6.1	23.6	-17.0	24.8	0.52	0.71	0.52	0.71
IL07-19334	41.3	58.0	38.4	68.2	1.6	24.7	15.3	1.1	0.40	0.69	0.39	0.61
IL07-20728	46.5	44.6	62.2	57.8	-5.4	38.8	-11.2	49.9	0.36	0.73	0.41	0.74
IL07-20743	44.9	50.0	53.8	60.2	10.4	31.7	2.3	15.6	0.50	0.67	0.47	0.67
IL07-21847	40.5	55.8	48.0	65.3	5.8	21.1	8.9	20.0	0.50	0.69	0.41	0.59
IL07-23420	42.6	58.1	53.3	59.5	-2.0	22.1	9.1	38.5	0.38	0.70	0.52	0.69
IL07-6861	51.6	49.6	66.5	66.8	-3.1	30.9	-16.4	33.1	0.45	0.70	0.41	0.76
IL08-34020	43.5	55.5	50.4	64.8	1.8	25.7	-6.2	2.1	0.39	0.69	0.46	0.60
IL99-26442	33.5	57.4	58.8	60.5	13.4	12.6	0.7	23.7	0.39	0.63	0.41	0.76
KY02C-1058-03	48.3	56.7	47.4	67.9	-1.0	16.8	7.7	19.9	0.36	0.72	0.45	0.69
KY02C-1076-07	47.6	57.8	47.9	65.9	-3.2	18.9	5.6	18.6	0.43	0.69	0.49	0.61
KY02C-1121-11	51.4	60.1	59.8	71.0	-12.7	15.5	-3.6	41.0	0.39	0.69	0.40	0.72
KY02C-1121-75	47.8	47.4	45.4	66.9	2.0	25.0	18.2	27.3	0.44	0.66	0.43	0.58
KY02C-1122-06	41.8	49.4	58.2	67.3	0.5	25.4	-14.9	16.1	0.49	0.75	0.40	0.71
KY02C-2215-02	49.6	51.6	40.9	62.0	-5.0	31.9	16.6	27.7	0.44	0.73	0.53	0.73
KY02C-3004-07	57.2	60.0	66.1	57.1	-15.5	12.5	-4.8	51.3	0.41	0.75	0.34	0.69
KY02C-3005-25	52.4	46.7	61.3	66.0	-3.1	32.8	-11.3	16.0	0.53	0.66	0.54	0.60
KY03C-1002-02	49.4	57.5	55.5	60.7	2.7	29.3	-2.4	42.0	0.41	0.69	0.45	0.71
KY03C-1192-37	52.2	58.0	68.8	75.4	-3.5	25.3	-15.3	7.8	0.45	0.73	0.41	0.62
KY03C-1195-10-1-5	55.6	59.8	57.8	59.2	-12.3	13.2	1.2	20.5	0.42	0.69	0.43	0.69
KY03C-1221-01	49.2	69.2	54.7	67.0	-5.2	11.6	-10.2	14.9	0.48	0.70	0.44	0.53
KY03C-1221-06	50.6	57.5	47.8	69.4	-6.6	21.7	11.0	8.0	0.38	0.64	0.45	0.58
KY03C-1221-22	40.9	55.9	55.4	67.5	2.0	21.8	-6.7	10.5	0.40	0.69	0.37	0.39
KY03C-1237-01	51.7	48.7	54.0	60.0	-8.7	29.1	8.8	50.2	0.39	0.73	0.50	0.70
KY03C-1237-15	40.4	53.3	50.2	64.7	10.9	17.4	7.8	20.8	0.43	0.74	0.43	0.69
KY03C-1237-32	47.2	60.2	59.7	68.7	3.0	10.2	-0.2	12.6	0.45	0.70	0.38	0.64
KY03C-2047-02	37.6	58.8	45.2	54.1	10.2	27.1	4.6	32.3	0.46	0.71	0.40	0.62
KY03C-2047-06	51.3	55.5	57.8	64.6	-6.8	30.0	-2.0	12.5	0.43	0.69	0.47	0.59
KY03C-2049-02	50.0	54.5	59.8	67.1	-7.6	13.3	-0.1	11.1	0.36	0.66	0.41	0.60
KY03C-2314-08	48.3	59.7	57.6	65.0	0.8	16.8	-3.5	42.7	0.44	0.73	0.45	0.67
KY03C-2399-02	45.4	60.7	51.5	67.2	-8.9	16.8	8.1	5.4	0.39	0.72	0.52	0.63
KY04C-1128-38-1-5	53.4	57.9	57.3	87.5	-8.9	24.4	2.9	-16.6	0.46	0.70	0.55	0.65
KY04C-2006-41-1-1	46.6	53.3	65.6	66.3	-4.1	34.2	-3.8	19.3	0.39	0.73	0.48	0.58
KY04C-2151-40	51.8	57.4	47.9	66.6	-4.5	24.0	8.5	27.6	0.50	0.75	0.44	0.64
KY04C-2151-41	56.2	60.2	57.4	67.3	-6.2	15.8	-5.1	25.9	0.37	0.65	0.43	0.62
KY04C-3006-33-14-3	57.4	53.0	59.2	61.5	-16.8	13.6	5.6	29.8	0.40	0.67	0.51	0.65
KY05C-1007-2-12-5	51.4	54.5	53.5	67.1	-4.4	23.5	2.9	28.0	0.44	0.72	0.37	0.58
KY05C-1105-42-20-1	50.0	52.9	34.3	78.4	-5.1	21.8	13.6	17.0	0.42	0.72	0.61	0.66
KY05C-1381-77-7-5	46.5	50.0	50.2	76.1	-1.7	25.7	5.0	15.4	0.50	0.69	0.58	0.72
KY05C-1617-17-17-3	46.3	62.7	47.2	73.2	0.4	17.4	13.0	4.5	0.37	0.72	0.46	0.59
KY06C-1003-139-8-3	57.1	51.5	41.4	67.8	-6.8	32.5	18.4	24.3	0.38	0.72	0.55	0.62
KY93C-1238-17-1	46.0	56.2	72.6	70.0	-2.3	32.6	-8.5	23.0	0.42	0.74	0.41	0.66
PEMBROKE	42.3	55.6	63.3	69.2	-0.5	21.2	-3.2	20.4	0.44	0.74	0.44	0.61
$\bar{x}$	47.3	55.4	54.5	66.4	-2.4	23.6	0.31	21.8	0.43	0.70	0.46	0.66
SE	0.87	0.60	1.10	0.88	0.95	0.96	1.23	1.85	0.01	0.005	0.01	0.01

Table A.5.5. Broad sense heritability (h^2) and 90% confidence interval (upper limit (UL), and lower limit (LL)) for agronomic traits across N environments and locations calculated from means squares from ANOVA. Heading date (Hd), anthesis date (Ad), height (H), test weight (Twt), yield (Y), vegetative biomass anthesis (Vba), vegetative biomass maturity (Vbm), harvest index (HI), % grain protein (Gp)

Trait	h^2	LL	UL
Hd	0.53	0.32	0.66
Ad	0.80	0.72	0.86
H	0.70	0.59	0.79
Twt	0.69	0.56	0.78
Y	0.16	-0.63	0.18
Vba	0.02	-0.34	0.33
Vbm	0.10	-0.32	0.34
HI	0.29	0.02	0.49
Gp	0.78	0.70	0.86

Table A.6.1. Genotype and entry key used for the principal component analysis.

Name	entry	Name	entry
FOSTER	**1**	**IL06-7550**	**21**
ALLEGIANCE	**2**	**IL07-6861**	**22**
KY93C-1238-17-1	**3**	**IL07-21847**	**23**
KY02C-3005-25	**4**	**IL07-23420**	**24**
KY03C-1237-15	**5**	**IL07-20728**	**25**
KY02C-1122-06	**6**	**IL07-20743**	**26**
KY03C-1192-37	**7**	**IL07-19334**	**27**
PEMBROKE	**8**	**KY03C-1237-32**	**28**
KY03C-1221-22	**9**	**KY03C-1002-02**	**29**
KY03C-1237-01	**10**	**KY02C-2215-02**	**30**
05222A1-1-2-1	**11**	**KY02C-1058-03**	**31**
07290A1-12	**12**	**IL01-11934**	**32**
KY03C-1195-10-1-5	**13**	**KY03C-2047-06**	**33**
KY04C-2006-41-1-1	**14**	**KY03C-1221-01**	**34**
KY06C-1003-139-8-3	**15**	**KY04C-2151-40**	**35**
KY05C-1617-17-17-3	**16**	**03207A1-7-3-1**	**36**
KY05C-1105-42-20-1	**17**	**03633A1-69-2-5**	**37**
0537A1-3-12	**18**	**04620A1-1-7-4**	**38**
KY04C-1128-38-1-5	**19**	**04719A1-16-1-1-7**	**39**
KY04C-3006-**33-14-3**	**20**	**05219A1-8-21-2-4**	**40**

Figure A.6.1. Genotypes in relation to the first two principal components in the control treatment in the warming study 2014 Lexington, KY. Principal component 1 (Prin1) is related to variation in traits related to NUpE, while principal component two is related to variation in NUtE and vegetative N content at maturity. The genotypes are represented by entry number. The genotype/entry number key can be found in table.

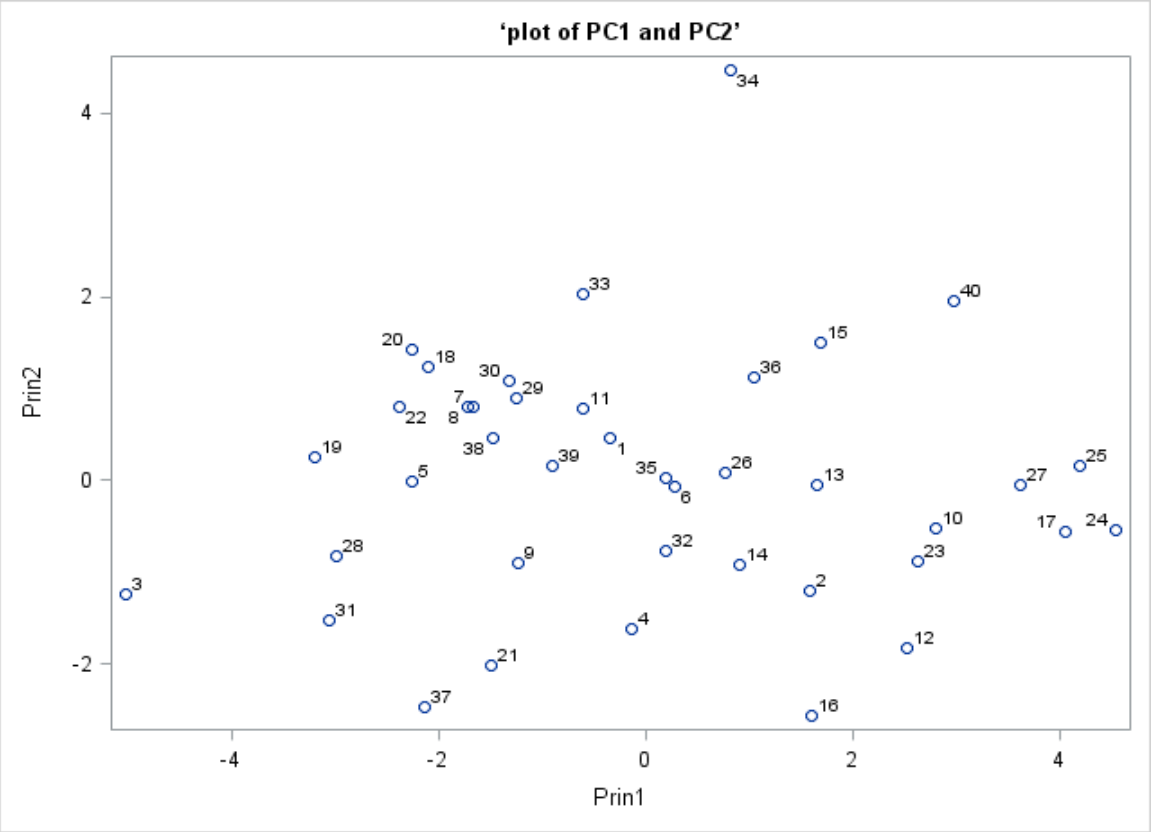
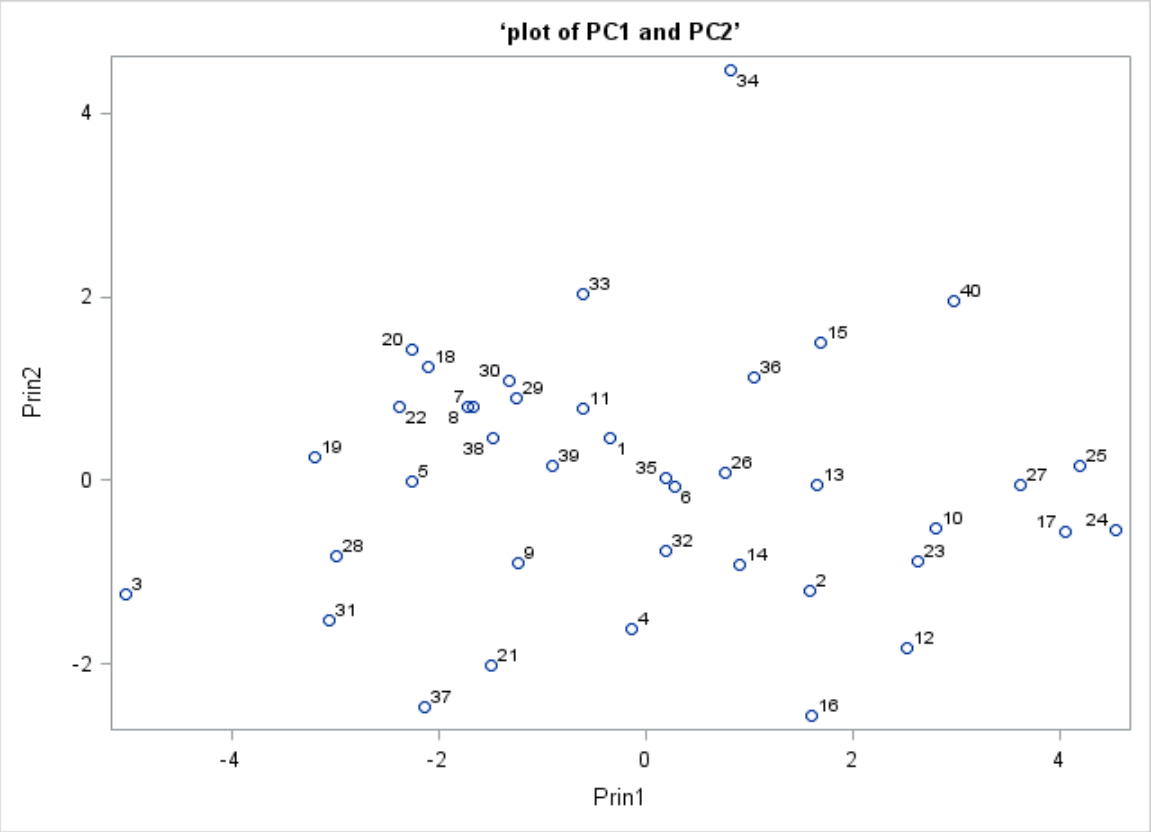


Figure A.6.2. Genotypes in relation to the first two principal components in the warmed treatment in the warming study 2014 Lexington, KY. Principal component 1 (Prin1) is related to variation in traits related to NUpE, while principal component (Prin 2) two is related to variation in NUtE and vegetative N content at maturity. The genotypes are represented by entry number. The genotype/entry number key can be found in table 6.12.



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